Research Program

Environmental Remediation

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The key driver for the Environmental Remediation Program (ERP) within ESD is to provide the scientific foundation needed for environmental remediation and water resources management. Over the last decade, it has become increasingly clear that if we are to face the environmental and water challenges of the future, we must view the system as a complex entity that includes the hydrosphere, geosphere, and biosphere. It has also become clear that these components are coupled and highly dynamic over various spatial and temporal scales. Since the U.S. Department of Energy (DOE) is responsible for the environmental health of over 140 contaminated waste sites across the United States, understanding the complexity of such natural systems is a prerequisite to successful stewardship of the DOE waste sites. As such, the majority of the enviromental research projects within the ERP focus on problems that are critical to the DOE waste sites. However, since many of the contaminants or closely related compounds found at these sites are also dominant at industrial waste sites, much of this research is also applicable to problems faced by the private sector and other government agencies. Because water resources and quality are particularly important for the vitality of water-stressed regions such as California, much of the water resources research performed in the ERP focuses on development of tools and techniques that will lead to better management of California water resources. A brief description of the environmental remediation and water resources programmatic areas of the ERP is given below.

ENVIRONMENTAL REMEDIATION

ESD scientists participating in the ERP conduct multidisciplinary environmental research using theoretical, characterization, modeling, and experimental approaches that range from the molecular to the field scale. The synergy offered by the ensemble of competencies within the ESD facilitates investigation of complex natural systems. Many of the projects within the ERP are associated with one of the following five themes:

- Development of advanced tools and approaches for characterizing biological, hydrological, and geochemical properties and processes at the molecular to field scale
- Development of tools to detect unexploded ordnance (UXOs)
- Improved understanding of complex natural systems and the impact of system complexity on contaminant distribution and remediation efficacy
- Improved ability to predict reactive contaminant transport in the subsurface
- Development of biological or chemical techniques capable of removing or sequestering contaminants

ESD scientists within the ERP program have developed many novel tools and techniques over the past few years that can be used to characterize properties and processes, and to monitor how they change in response to environmental perturbations. The newly developed Virtual Institute of Microbial Stress and Survival (VIMSS), based at Berkeley Lab, seeks to identify stress-response pathways of microbes important for environmental remediation. ERP scientists associated with VIMSS have determined the signature genes for sulfate-reducing bacteria, developed techniques for large-scale biomass production needed for cell analysis, and have developed a community resource for comparative microbial genomics. ERP scientists associated with VIMSS have also successfully used microarray techniques to characterize the phenotype of bacterial strains important for environmental remediation and have used PLFA techniques to determine the phenotypic response of cells to environmental stressors. High-density DNA microarray technologies were used by ERP researchers to detect large numbers of microorganisms within complex environmental environments, and to accurately monitor changes in the composition of microbial populations during laboratory- and field-scale studies of uranium (U) bioremediation.

Collectively, these studies improve our understanding of microbiological properties and processes, which is critical for designing sustainable contaminant bioremediation treatments.

ERP scientists are also involved in the Center for Environmental Kinetics Analysis, a collaborative effort exploring what molecular approaches and insights can be developed to extrapolate rates of environmentally important reactions across a variety of scales. In particular, ERP scientists are investigating the rates of formation and the topology of oxyhydroxide and silicate precipitates from the molecular to the nanoscale using synchrotron techniques, with a goal of understanding precipitate formation and reactivity. Scientists involved in this Center are also investigating mineral dissolution and precipitation rates under biotic and abiotic conditions via synchrotron-based experiments, using microfluidic reactive flow devices and x-ray spectroscopy methods.

Many advances have recently been achieved by ERP scientists in the use of high-resolution geophysical data for characterizing and monitoring subsurface systems. Stochastic joint inversion techniques were developed to combine geophysical and hydrological data for the estimation of hydrological properties at the Hanford Site in Washington, and to estimate fracture zonation at the Oak Ridge National Laboratory in Tennessee. During the past two years, ERP scientists have discovered that noninvasive geophysical techniques could be used at the laboratory scale to monitor biomineralization and gas production associated with bioremediation processes. In related studies, surface geophysical data were used to indicate the change in clay mineralogy caused by bioremediation at the field scale, and tomographic geophysical data were used to monitor amendment distribution associated with a Cr(VI) bioreduction study at the Hanford Site. These studies reveal the potential of high-resolution geophysical data sets for providing the hydrogeological characterization information that is important for predicting contaminant transport, or for noninvasively monitoring system transformations during remedial processes.

Significant advances have also recently been made by ERP scientists in the development of geophysical tools that can detect and extract essential information about buried metallic objects—information that is essential for identification and discrimination of unexploded ordnance (UXO). Through research that received the Strategic Environmental Research and Development Program (SERDP) Project of the Year Award, ERP scientists have developed an active electromagnetic (AEM) field prototype system that can obtain estimates of the location, size, shape, and metal content of a buried metallic object in the presence of other metallic clutter.

Novel isotopic approaches were successfully developed and applied by ERP scientists to identify the source of contaminants at DOE waste sites. Precise U isotopic approaches were used to analyze the pore water of subsurface sediment samples at the T-Waste Management Area (WMA) of the Hanford Site in Washington, and to unravel the history of U contamination there. Isotopic analysis of U and strontium isotopes were used to trace the source of U from the site to the Hanford Reach of the Columbia River, and to monitor how the flux varies with seasons. ERP scientists have streamlined a nitrate isotopic analysis technique and used the technique to study the nitrate distribution and origin at several Hanford vadose zone and groundwater sites. Such novel isotopic approaches are critical to establishing both an understanding of complex transport in natural systems and for establishing remediation strategies and responsibilities.

Laboratory studies conducted by ERP scientists have revealed phenomena that have significant implications for designing and sustaining environmental remediation approaches at the Hanford site. Column studies, designed to mimic the leakage of highly saline and alkaline radioactive waste solutions from Hanford storage tanks into sediments, revealed a significant pH reduction and colloid formation at the plume front. These observations suggest the importance of considering plume-front phenomena for predicting the behavior of contaminants in the Hanford subsurface. Column studies were also used to investigate the conditions that control long-term stability of bioreduced U. These studies indicated that at 100 to 500 days after U reduction, U was reoxidized and solubilized even in the presence of a microbial community capable of reducing U(VI). This study suggests that *in situ* U remediation using organic carbon-based reductive precipitation can be problematic in sediments when uranyl carbonates are stable.

ERP scientists have recently assessed the potential for immobilizing and detoxifying chromium (Cr)-contaminated groundwater at the Hanford 100H site using lactate-stimulated bioreduction. Microbial, geophysical, and geochemical analysis of groundwater, coupled with stable isotope monitoring, permitted accurate tracking of microbial processes during this field treatabilty study, and confirmed that Cr(VI) was successfully removed from groundwater at the Hanford 100H Site. Such studies reveal the benefits of interdisciplinary approaches for investigating and manipulating complex earth systems.

WATER RESOURCES AND QUALITY

To optimally manage our water resources, ERP scientists are developing tools and approaches that can be used to measure, understand, and predict the flow, transport, and residence times of water, nutrients, and contaminants through natural water systems. With a population of over 30 million people, an agricultural economy based on intensive irrigation, and large urban industrial areas, California is highly dependent on water for its vitality and productivity. Consequently, much of the water research within the ERP is focused on problems important to this state.

ERP scientists have developed a variety of tools to characterize hydrological, geochemical, and biological properties and processes needed for managing water resources. Motivated by the concern about endocrine-disrupting compounds in recycled water, ERP scientists have developed a prototype biosensor capable of assessing estrogenic compounds. These scientists have successfully used the biosensor to assess water from various wastewater treatment and natural systems. Several logging tools have also been developed by the ERP this year and applied to watershed studies, including the electrical conductivity sensors used to identify high-salinity zones in agricultural areas, and heat-based sensors used to indicate flow velocities.

System-level studies are being carried out to understand complex hydrological and ecological phenomena. ERP scientists are investigating the San Joaquin River in the Central Valley of California to examine how non-point-source pollution discharge associated with agricultural activities impacts water quality. Their investigations involve field measurements to determine mass balance of algae and nutrients within the system. Understanding the conditions that give rise to unsaturated flow below a streambed in Sonoma County, California, and the impact that this may have on well operations in the area, is being investigated by ERP scientists using advanced measurement and modeling techniques. ERP scientists are also actively investigating water-balance and water-quality issues within several California counties, including Merced County. Remediation approaches have also been recently developed by ERP scientists working on water resources projects. Our scientists developed an algal-bacterial selenium removal process deployed within the agricultural drainage area of the western San Joaquin Valley to remove nitrate and selenium from irrigation drainage. An important component of many of the water resource projects is the development of computer-based decision support systems to enhance environmental monitoring and management of water systems.

ERP PROGRAM MANAGEMENT AND TECHNICAL ASSISTANCE

ERP continues to be the Natural and Accelerated Bioremediation Research (NABIR) Program Office for the Office of Science. The NABIR Program Office maintains the dynamic NABIR Web home page (www.lbl.gov/NABIR/) with links to investigators, program element managers, science team leaders, recent publications, annual meeting registration, calls for proposals, review documents, and other Web sites. In addition, the NABIR Program Office also organizes the NABIR annual investigators meeting, with more than 150 participants and sessions for posters, presentations, and breakout sessions.

ERP scientists also manage the Environmental Program at the Advanced Light Source (ALS), as described by http://esd.lbl.gov/ALS_environmental/index.html. This program is designed to assist environmental researchers in gaining familiarity with and access to the ALS, and in assisting with environmental investigations at the ALS. Many of the environmental investigations in this program focus on understanding how and what microbiological and geochemical species are distributed relative to contaminants and within natural geological materials, which processes occur, and the rates at which they occur. The program provides support across four beamlines, enabling a range of measurement support scales from nanometers to centimeters.

ERP scientists provide technical assistance to DOE Environmental Management International projects. The overall objectives of this program are to use foreign sites as analogues to improve the capability of DOE's conceptual and numerical models for predicting radionuclide transport and impact, and to improve DOE remediation technologies. Recent projects have focused on helping in the design of site characterization, radionuclide transport modeling, and remediation technologies at the Kurchatov Institute in Russia, the Chernobyl Exclusion Zone in Ukraine, and the Ezeiza Atomic Center in Argentina.

PARTNERS AND FUNDING

ERP receives much of its support for environmental research from DOE programs in the Office of Science, Office of Biological and Environmental Research. These programs include the Natural and Accelerated Bioremediation Research (NABIR) and the Environmental Management Science Program (EMSP) of the Environmental Remediation Sciences Division, and from the Genomics: GTL Program. DOE's Office of Environmental Management supports the International Program technical assistance and also supports some of the research performed at the Hanford, Washington, site. Research associated with unexploded ordnance is supported by SERDP. Much of the California water resources and quality projects are supported by the CALFED Bay-Delta Program, Department of Water Resources, and the U.S. Bureau of Reclamation. Support for ERP projects is also provided by NASA, the Department of Defense, the Department of Homeland Security, Cal-EPA, other DOE Labs, the Berkeley Lab LDRD Program, Sonoma County Water Agency, U.S. Army, DHS, UC Berkeley, Panoche Drainage District, and the U.S. Bureau of Land Management.

Real-Time PCR with Reverse Transcription for Quantification of Chlorinated Solvent Degradation

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RESEARCH OBJECTIVES

The purpose of this project is to develop and apply culture-independent molecular approaches for characterizing microbial communities capable of degrading chlorinated solvents in subsurface aquifers.

APPROACH

Our approach is to apply quantitative real-time polymerase chain reactions (qPCR) and reverse transcription to quantify the occurrence and expression of dehalogenating species and genes in laboratory enrichments under varying conditions. Whole-genome microarrays will then be applied to query the transcriptome of *Dehalococcoides* strains.

ACCOMPLISHMENTS

Functional-gene expression analysis using quantitative PCR

By combining qPCR with reverse transcription, we can accurately monitor the expression level, defined as the ratio of mRNA to DNA, of any expressed gene. We have used this technique to study changes in the expression of the *Dehalococcoides* functional reductase gene, *tceA*, within the ANAS mixed community, in response to growth condition perturbations. We found that expression of the *tceA* gene dramatically increases after cells are exposed to trichlorylethylene (TCE) or isomers of dichlorylethylene (DCE), but not perchlorylethylene (PCE) or vinyl chloride (VC). The *tceA* gene expression response did not depend on the sum concentration of TCE and DCE when they were present in environmentally relevant concentrations (≥1.8 μM), nor on the concentration of electron donor above threshold concentrations (≥ 17 nM H_2), or the presence of common microbial electron acceptors (sulfate, sulfite, thiosulfate, nitrate, nitrite, or fumarate). Incubation temperature, however, had a substantial effect on *tceA* expression, with *tceA* expression at 30°C more than 10-fold higher than at 14°C.

Cooperation and interactions between Dehalococcoides strains

We used qPCR to study the *Dehalococcoides* population within an enrichment culture, using primers for three different functional reductase genes and the *Dehalococcoides* 16S rDNA. The data suggest that the enrichment culture contains two cooperating *Dehalococcoides* strains, which in turn contain reductases with different functions in roughly a 1:2 ratio; and that the relative amounts of the two strains can be dramatically altered by adjusting solvent exposure conditions.

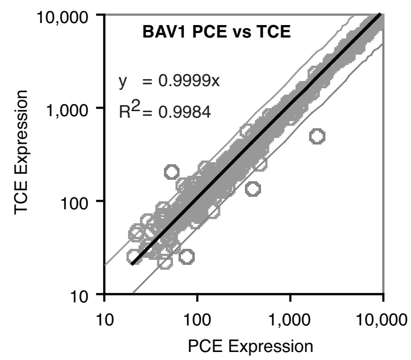

Figure 1. Comparison of microarray fluorescence intensity after 8-hour PCE or TCE exposure for the 618 genes that had significant signal:noise ratios under both conditions. Faint lines indicate 2-fold difference, while the solid line is a linear fit to the data. BAV1 cells were grown on VC and exposed just before exhaustion of VC. BAV1 is a strain of *Dehalococcoides sp.* that utilizes vinyl chloride for growth by means of dehalorespiration.

Analyzing genomics and transcriptomics using whole-genome microarrays

An Affymetrix whole-genome microarray for *D. ethenogenes* 195 was designed in collaboration with Gary Anderson of Berkeley Lab and Professor Stephen Zinder of Cornell University. The queried transcriptome produced statistically significant hybridization signal on roughly 40% of the 1,600-gene chip: 725 genes showed expression after either PCE or TCE exposure, and 618 genes showed expression under both conditions. The reproducibility between TCE- and PCE-exposed cells was excellent (Figure 1), with an R-2 value of 0.9984.

RELATED PUBLICATIONS

Johnson, D.R., P.K.H. Lee., V.F. Holmes, and L. Alvarez-Cohen. An internal reference technique for quantifying specific mRNAs by real-time PCR with application to the *tceA* reductive dechlorination gene. Applied and Environmental Microbiology (in press), 2005.

Johnson, D.R., P.K.H. Lee, V.F. Holmes, and L. Alvarez-Cohen. Environmental factors affecting the expression of the *tceA* reductive dechlorination gene in an anaerobic microbial enrichment culture. Applied and Environmental Microbiology (submitted), 2005.

ACKNOWLEDGMENTS

This work was supported by Laboratory Directed Research and Development (LDRD) funding from Berkeley Lab, provided by the Director, Office of Science, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Whole Genome Transcriptional Analysis of Toxic Metal Stresses in *Caulobacter Crescentus*

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RESEARCH OBJECTIVES

Caulobacter crescentus is an extremely ubiquitous organism with a distinctive ability to survive in low nutrient environments. In this study, we exposed the *Caulobacter crescentus* cells to five heavy metals (chromium, cadmium, selenium, lead, and uranium) and analyzed genome-wide transcriptional activities. The understanding of resistance pathways can provide important insight and knowledge for environmental restoration.

APPROACH

Transcriptional regulation was measured using a *Caulobacter* Affymetrix GeneChip array custom designed by the McAdams Lab (Stanford, California). In addition to the multiple probes for all predicted coding regions, probes were tiled for both strands, encoding all hypothetical proteins plus all intergenic regions. This feature makes it possible to detect all transcripts without prior knowledge and bias, including untranslated regulatory RNAs and antisense transcripts (transcripts from the opposite strand of a predicted open reading frame).

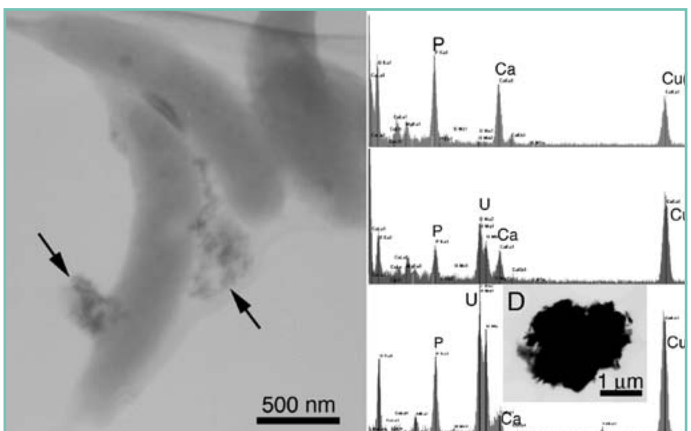

Figure 1. Extracellular uranium-bearing precipitates. The EDX showed the precipitates contained uranium, phosphate, and calcium.

We have also used electron microscopy (EM) to visualize *Caulobacter crescentus* cells under 200 µM uranium stress for 30 minutes and found that uranium was associated with cells extracellularly. Energy Dispersive X-ray (EDX) analysis was carried out to determine elemental compositions of uranium-bearing solid phases associated with cells.

RESULTS

In addition to the unexpected finding that *C. crescentus* CB15N is tolerant to high levels of uranium, our studies—combining physiology observation, transcriptional measurement, and imaging analysis—clearly showed that *Caulobacter* formed a calcium-uranium-phosphate precipitate extracellularly (Figure 1). This observation was consistent with a limited response to oxidative stress such as that seen with other metals and also with the up-regulation of a secreted calcium-dependent phytase domain protein, which may serve as a nucleation site for uranium precipitation. The strategy of lowering intracellular metal concentration was also present in cadmium and chromium response. Efflux pumps were up-regulated under cadmium stress. *C. crescentus* does not seem to have a specific extrusion mechanism for chromium; however, the cells down-regulated a sulfate transporter, which may reduce the uptake of chromate.

Our data have also clearly demonstrated the importance of interrogating the whole genome on both strands. We have identified at least six antisense transcripts that are differentially regulated specific to metals, which, as either proteins or RNAs, may play an import part in the response model. Using knockout mutants, we also confirmed regulatory roles played by a pair of two-component signal transduction proteins under uranium stress.

SIGNIFICANCE OF FINDINGS

The principal response to most metals was protection against oxidative stress (up-regulation of manganese-dependent superoxide dismutase, *sod*A), while glutathione S-transferase, thioredoxin, glutaredoxins and DNA repair enzymes responded specifically to cadmium and chromate. Cadmium and chromium stress response also focused on reducing the intracellular metal concentration, with multiple efflux pumps being employed to remove cadmium while a sulfate transporter was down-regulated to reduce nonspecific uptake of chromium. The function of a two-component signal transduction system involved in uranium response was confirmed by knockout mutants. In addition, several differentially regulated transcripts from regions previously not known to encode proteins were identified, demonstrating the importance of evaluating the transcriptome using the whole genome.

RELATED PUBLICATION

Hu, P., E.L. Brodie, Y. Suzuki, H.H. McAdams, and G.L. Andersen, Whole-genome transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*. Journal of Bacteriology, 187(24), 8437-49, December 2005. Berkeley Lab Report LBNL-59011.

ACKNOWLEDGMENTS

This study was funded by *Department of Energy, Genomes to Life: Microbial Cell Program*. This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Berkeley National Laboratory, under Contract No. DE-AC03-76SF00098.

Berkeley Lab
http://www-esd.lbl.gov/

16S rDNA Microarray for Microbial Community Analyses in Air, Soil, and Water

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RESEARCH OBJECTIVES

Knowledge of the microbial community structure, including the predominant species composition and shifts in population dynamics, is necessary for understanding the effect of any perturbation on a natural ecosystem. The resulting mixed amplicons can be quickly, but coarsely, typed into anonymous groups using molecular techniques such as restriction fragment length polymorphism. Subsequent sequencing allows application of taxonomic nomenclature to the groups, but requires additional labor to physically isolate each 16S rDNA type and does not scale well for large studies. Instead, hybridizing polymerase chain reaction (PCR) products to a high-density universal 16S rDNA microarray allows rapid taxonomic classification of community members. In this study, we asked, "Which method is more comprehensive in cataloging an environmental prokaryotic 16S amplicon community, clone-and-sequencing or microarray?"

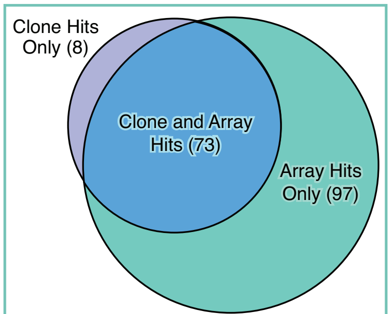

Figure 1. Comparison of bacterial groups detected by either 16S Microarray or 16S Clone Library

APPROACH

Molecular approaches aimed at broad prokaryotic environmental detection routinely rely upon classifying heterogeneous nucleic acids amplified by universal 16S rDNA PCR. Cloning-and-sequencing the PCR products has been the general method of sampling the DNA types, but does not scale well for large studies. Instead, hybridizing PCR products to a universal 16S rDNA microarray allows a more rapid evaluation. We have developed a high-density microarray system to accurately measure the key microbial components in air, water, and soil environments. Unique regions of DNA within gene sequences of a 16S ribosomal RNA small subunit are used to identify specific organisms. A minimum of 11 oligonucleotide probes (25-mers) are used in combination to identify, in parallel, any of over 9,000 distinctive species or taxa on a 500,000-probe, high-density microarray. The combinatorial approach of multiple probes has clear advantages over a single probe for the identification of a target sequence. Broad-range bacterial and archaeal 16S primers that target conserved areas at the 5′ and 3′ ends of the 16S rRNA gene are used to amplify 1,400–1,500-bp fragments for analysis. This 16S microarray system was used to measure the microbial diversity of aerosols collected from a biosurveillance network—subsurface sediment contaminated with uranium and deep subsurface water from a South African gold mine.

RESULTS

In each sample, the array revealed a greater number of taxonomic groups of bacteria than the corresponding clone library. This result was expected, since nonasymptotic rarefaction curves demonstrated that the clone libraries were only a partial sample of the total sequence diversity. To validate the presence of the bacterial groups, which were detected only by the array, taxon-specific primers were created for the aerosol sample. The resulting sequences verified the array detection of probe-sets corresponding to specific bacterial groups. Entire phyla, including *Nitrospira* and *Spirochaetes,* would have been overlooked if the clone library were the sole source of taxonomic sampling. In addition, sample-to-sample variation in probe hybridization intensity of a previously identified airborne organism, *Pseudomonas oleovorans*, was independently confirmed by quantitative, real-time PCR amplification, with species-specific primers using the genomic DNA from the concentrated aerosol samples.

SIGNIFICANCE OF FINDINGS

A hybridization-based approach using oligonucleotide microarrays can be applied for the fast and parallel detection of large numbers of microorganisms from complex environmental samples. Constrained by the numbers of different oligonucleotide probes that can be spotted within a given area, most arrays have been designed to measure a selected subgroup of organisms that have been hypothesized to play critical roles within a larger community. We developed an approach to leverage the vast amount of 16S sequence data available in public databases and search for unique sequences that can be used for identification. Compared with sequencing a 16S rDNA clone library, the microarray was unable to recognize novel prokaryotic families, but could identify greater diversity from organisms with similarity to existing sequence (Figure 1).

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Berkeley National Laboratory under Contract No. DE-AC03-76SF00098, and was funded in part by the Department of Homeland Security under Grant No. HSSCHQ04X00037.

Development of Environmental Biosensors for Endocrine Disrupters

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RESEARCH OBJECTIVES

Water recycling is an important strategy for management of this scarce resource, but concerns about residual contamination in recycled water remain a limiting barrier in implementing water conservation through water recycling. Concerns have developed around endocrine disrupting compounds (EDCs) in recycled water because of their ability to mimic hormones involved in many biological processes, including immune function, reproduction, growth, and control of other hormones. EDCs are hormonally active at small concentrations (parts per billion or trillion). The list of EDCs found in water is steadily growing and includes many common agricultural, industrial, and household chemicals and their degradation products. Significant sources of EDCs include both synthetic chemicals like pesticides and those produced naturally by plants and animals. The objective of this work is to develop analytical capabilities to characterize the diverse sources of EDCs in urban and agricultural sources to evaluate the connection between EDCs and pesticides, and to develop biosensors to detect EDC activity in natural sources.

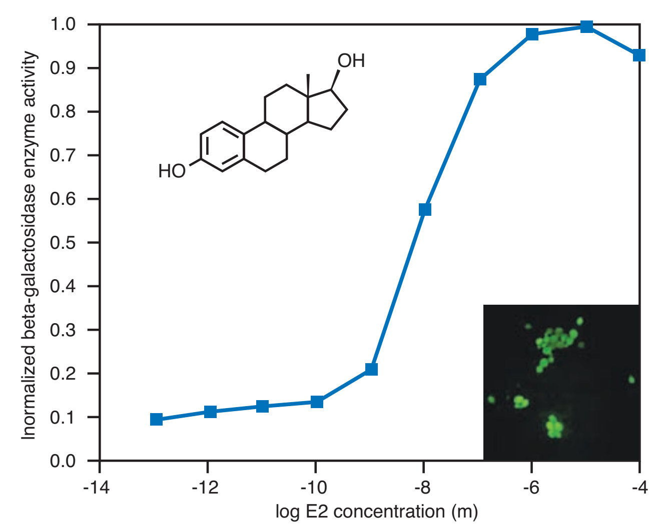

Figure 1. Dose-response of β-galactosidase enzyme production when the estrogen-responsive yeast strain RMY/ER-ERE is exposed to the hormonal estrogen 17β-estradiol (E2). Inset is the chemical structure of E2 and the fluorescent response of live yeast cells to E2. Other estrogenic compounds, including some EDCs, may behave in a similar manner, eliciting a similar response.

APPROACH

Traditional analytical techniques (gas chromatography, gas chromatography/mass spectrometry, high-pressure liquid chromatography) were used for calibration and verification of bioassays. Analytic capabilities were developed for hormones in both sediment and water to improve sensitivity and modify the techniques for use in an automated system. Enzyme-linked immunosorbent assay (ELISA) kits were used for analysis of estrogenic chemicals in water, wastewater, wastewater solids, and sediment. Chromogenic and fluorescent bioassays were developed to monitor estrogenic activity in water samples, and the response was studied using microscopy and spectroscopy to evaluate cell structural changes, viability, and response (Figure 1).

ACCOMPLISHMENTS

A prototype biosensor system was developed to measure the cellular response of luminescent microbes, providing the hardware necessary to use a luminescent EDC biosensor using fluorescence. Extensive bioassay development was performed on time-lapsed EDC activity response in live estrogen-sensitive yeast cells. This fluorescence assay was used in conjunction with Fourier-transform infrared (FTIR) spectromicroscopy, a noninvasive technique used for collecting real-time data on live cells. Employing these methods, we collected dose-response and abundant complementary data on multiple intercellular mechanisms (Figure 1).

SIGNIFICANCE OF FINDINGS

The biosensors developed provide a novel tool for assessing estrogenic compounds and surfactants. We have applied the biosensors to waters from agricultural systems and will apply them to water from the San Joaquin River Basin, and various wastewater treatment systems. Additionally, the sensors will be applied in wastewater treatment plants to demonstrate their usefulness for management of EDC degradation in wastewater treatment processes.

RELATED PUBLICATIONS

Campbell, C.G., S.E. Borglin, W.T. Stringfellow, F.B.Green, and A. Grayson, Review of bioassays for monitoring fate and transport of estrogenic endocrine disrupting compounds in water. Critical Reviews in Environmental Science and Technology (submitted), 2005.

Campbell, C.G., S.E. Borglin, W.T. Stringfellow, F.B.Green, and A. Grayson, Biologically based sensors for endocrine disrupting compounds in water. Conference Paper, American Society of Civil Engineers, Alaska, May 15–20, 2005.

ACKNOWLEDGMENTS

This work was supported by Laboratory Directed Research and Development (LDRD) funding from Berkeley Lab, provided by the Director, Office of Science, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Environmental Lipidomics of Microbial Community Structure and Function

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RESEARCH OBJECTIVES

Phospholipid Fatty Acid (PLFA) analysis has become an interesting and valuable tool for determining the microbial community structure in soils, water, and other environmental samples with complex microbial communities. During PLFA analysis, phospholipids from cell membranes of microorganisms are extracted and used in determining the predominant types of microorganisms in the system, give indications of the physiological status of the microbial community, and also provide a means for estimating the microbial biomass. This type of information is valuable in evaluating changes in community structure and status during remediation or treatment activities, and can also be useful in evaluating the microbial status of natural systems. Our research focuses not only on identifying microbial community structure in the environment, but also applying this technique to look at stress response of pure cultures of metal-reducing bacteria. These bacteria are exposed to stressors in the environment that affect viability as well as the efficiency of metal reduction during the bioremediation processes. Because phospholipids are part of the cell membrane, changes in lipid composition are one of the first phenotypic responses to stress and give insight into cell response and survival mechanisms.

APPROACH

To determine the lipid response to stress, we grew both *Desulfovibrio vulgaris* and *Shewenella oneidensis* in batch culture and exposed them to a variety of stressors, including cold, heat, pH, salt, nitrate, and oxygen. The phospholipids were extracted from the cultures at different time points to determine how the cell membrane responded to stress and to determine if specific fatty acid patterns can be used as an indicator of phenotypic response to stress analysis.

ACCOMPLISHMENTS

To date, approximately 40 *Desulfovibrio vulgaris* and 5 *Shewenella oneidensis* stress experiments have been completed. The results show that the lipid response is varied and highly dependent on stress conditions and organism type. For example, during salt stress, *Desulfovibrio vulgaris* increases its amount of lipid per cell, and at the same time increases its proportion of saturated lipids (see Figure 1). During oxygen stress of *D. vulgaris*, no growth occurs in the cells, but the PLFA analysis shows also that no significant death occurs, and there is little or no change in the lipid patterns or total amount of lipids in the culture. For *Shewenella oneidensis*, many genes involved in production of saturated and/or branched-chain fatty acids are affected by both temperature and salinity. During salt stress, the cells adapt their membrane fluidity to external conditions by increasing the proportion of unsaturated lipids and decreasing the relative amount of unsaturated lipids.

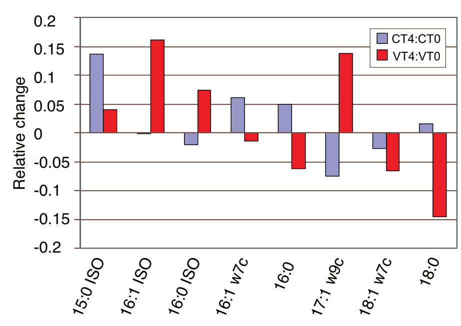

Figure 1. Relative changes in the 8 major types of PLFA after NaCl stress

SIGNIFICANCE OF FINDINGS

This research documents the phenotypic response of cells to environmental stressors. Continued work will be focused on linking the PLFA phenotypic responses to genetic pathways. This research is expected to increase the ability to identify stress responses in environmental samples.

RELATED PUBLICATION

Borglin, S., T. Hazen, D. Joyner, R. Huang, N. Katz, E. Alm, and A. Kazakov, Phospholipid fatty acid analysis as phenotypic indicators of common stress response pathways in *Desulfovibrio vulgaris* and *Shewanella oneidensis*. ASM General Meeting, Atlanta, Georgia, June 8, 2005.

ACKNOWLEDGMENTS

This work was part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program: Genomes to Life (GTL) through Contract No. DE-AC03-76SF00098 between Berkeley Lab and the U.S. Department of Energy.

PHENOTYPIC MICROARRAY ANALYSIS FOR PHENOMICS AND PATHWAY ANALYSES IN ANAEROBES

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RESEARCH OBJECTIVES

Phenotypic Microarray™ analysis is a recently developed analytical tool to determine the phenotype of an organism. This technique can be useful in understanding the growth changes of an organism when changing medium, temperature, or adding a stressor, or when testing mutant strains. The tool, which is commercially available from Biolog™ (Hayward, CA), consists of an array of 20 plates. The first eight plates test a variety of metabolic agents, including electron donors, acceptors, and amino acids. Plates 9 and 10 cover pH and osmotic stressors, while plates 11–20 contain a variety of inhibitors, including toxic agents and antibiotics. In total, the plates simultaneous test nearly 2,000 independent conditions on a single bacteria culture.

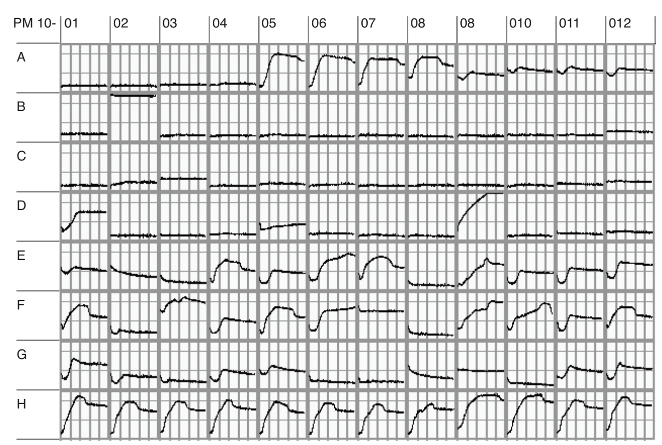

Figure 1. Growth of *Desulfovibrio vulgaris* in phenotypic microarray plate 10 (PM10). This plate measures the effect of pH on growth. Each plot represents growth in a different pH condition, the horizontal axis representing time (7 days), the vertical axis representing increase in turbidity, which is correlated with growth.

APPROACH

Techniques have been developed at Berkeley Lab for using these plates under anaerobic condition, to enable culturing of an obligate anaerobe, *Desulfovibrio vulgaris*. To accomplish this task, plates were set up in an anaerobic chamber and heat-sealed in polyethylene bags containing an anaerobic sachet. This technique permitted maintenance of anaerobic conditions in the plates for up to a week. Growth of the cells was measured by the increase in turbidity of the cells, which was correlated with both optical densities at 600 nm and total cell counts. Preconditioning of the cells and specialized media preparation are required for the different types of plates to obtain a valid phenotype.

ACCOMPLISHMENTS

The plates have been successfully used to characterize the phenotype of the *Desulfovibrio vulgaris* American Type Culture Collection (ATCC) strain (see Figure 1). The plates are currently being applied to mutant strains to provide rapid screening of mutant phenotypic changes needed for rapid pathway analyses and modeling. Several method-development obstacles have been overcome, including optimization of the plate-sealing technique, density of the inoculated organisms, and false positive and negative results from either excess or deficient inoculum.

SIGNIFICANCE OF FINDINGS

This technique will allow the production of high-volume and high-quality data related to the effect of targeted mutations and environmental stressors on bacterial cultures. This array, which will provide a unique tool for the study of bacterial stress and survival, is currently being applied to metal-reducing bacteria in soil.

RELATED PUBLICATION

S. Borglin, T. Hazen, J. Carlson, J. Wall, D. Joyner. Phenotypic microarray analysis of *Desulfovibrio vulgaris*. ASM General Meeting, Atlanta, Georgia, June 8, 2005.

ACKNOWLEDGMENTS

This work was part of the Virtual Institute for Microbial Stress and Survival supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program: Genomes to Life (GTL) through Contract No. DE-AC03-76SF00098 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.

Bioreoxidation of Uranium from the Oak Ridge Y-12 Site: Microbial Community Structure and Function

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Tetsu Tokunaga, Jiamin Wan, Terry Hazen, Gary Andersen, Paul Richardson, Don Herman[1], and Mary K. Firestone
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RESEARCH OBJECTIVES

Uranium contamination is an unwanted legacy of the Cold War era. When uranium mining and processing for nuclear weapons and fuel were at their peak, uranium-containing wastes accumulated, resulting in a multitude of contaminated sites around the world. Uranium remediation strategies in recent years have focused on containment, minimizing migration of uranium in groundwater to prevent infiltration into surrounding water courses and potable water supplies.

One promising approach to minimizing uranium migration is to catalyze the reduction of soluble U(VI) to the less soluble U(IV). This process can be accelerated by the action of indigenous microorganisms fuelled through addition of exogenous carbon. Organic carbon addition stimulates biomass and microbial activity in these typically nutrient-poor environments, and has a profound impact on microbial community composition. The focus of our work is to use novel high-density DNA microarray technology to accurately monitor changes in composition of microbial populations during lab and field-scale studies of uranium bioreduction and observed reoxidation/remobilization.

APPROACH

To determine if the remobilization of U(VI) was associated with alterations in microbial populations, we have used a novel high-density oligonucleotide-microarray-based approach, which permits simultaneous monitoring of the dynamics of over 9,000 distinguishable prokaryotic taxa/units (OTUs) (Figure 1). To identify dynamic groups of organisms during biostimulation, we have applied hierarchical clustering methods combined with global graphical representation methods. To validate the high-density array approach, we analyzed identical samples, using a more common clone-library approach in addition to confirmatory tests using quantitative polymerase chain reactions (PCR). This is the first application of high-density array technology in profiling complex microbial communities such as those in soils or sediments.

ACCOMPLISHMENTS

Array analysis of the contaminated sediment confirmed the presence of most clone sequence types detected using conventional methodology. In addition, array analysis also indicated the presence of many bacterial families not detected by cloning, including those of importance for uranium reduction (e.g., *Geobacteraceae*). PCR with primers specific for *Geobacteraceae* confirmed this finding. Array analysis of bacterial communities during a laboratory-scale remediation simulation permitted time- and cost-effective monitoring of the dynamics of over 9,000 groups of bacteria. By using hierarchical clustering and principal component analysis, it was possible to readily identify those organisms responding with treatment or over time.

Lactate infusion into columns resulted in a significant change in bacterial populations, and following an initial period of successful uranium immobilization/reduction, we observed a remobilization of uranium, despite an adequate supply of lactate and suitable redox conditions. However, array data demonstrated that bacteria capable of uranium reduction had not decreased in quantity; therefore, a loss of this functional group was not considered the primary reason for the remobilization of uranium.

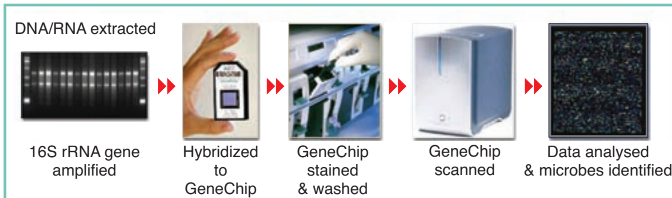

Figure 1. Flow schematic for high-density DNA microarray analysis of environmental microbial communities

SIGNIFICANCE OF FINDINGS

This is the first application of high-density microarrays in analysis of complex microbial communities. We have also demonstrated the ability to accurately track complete populations of bacteria using this array and have shown that loss of microbial functional groups was not a primary cause of uranium remobilization. Bacterially mediated carbonate accumulation has been identified as a possible driver of uranium remobilization, and we are currently investigating further biological and geochemical explanations for this significant observation.

PUBLICATIONS

Wan, J., T.K. Tokunaga, E.L. Brodie, Z. Wang, Z. Zheng, D. Herman, T.C. Hazen, M.K. Firestone, and S.R. Sutton, Reoxidation of bioreduced uranium under reducing conditions. Environmental Science and Technology, 39, 6162-6169, 2005.

ACKNOWLEDGMENTS

This work was supported by the Director, Office of Science, Office of Biological and Environmental Research, Environmental Remediation Sciences Division, Natural and Accelerated Bioremediation Research (NABIR) Program; and the Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. Support was also provided by GeoSoilEnviroCARS, Advanced Photon Source (APS), Argonne National Laboratory.

Ecogenomic Analysis of Very Deep Subsurface Environments as Analogues to Life on Mars

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RESEARCH OBJECTIVES

Terrestrial subsurface ecosystems have been proposed as analogues to life on other planets. Subsurface environments may provide habitable niches, protecting microorganisms from surface-related stress such as ultraviolet radiation, although at a cost. These environments are typically isolated from standard terrestrial energy economies (i.e., those revolving around solar energy): temperatures in the deep subsurface can reach 60°C. As such, microbes inhabiting these deep-subsurface ecosystems must adapt to such harsh conditions and be capable of obtaining energy by other means. By studying such isolated ecosystems, we hope to better understand the thresholds of life and determine the feasibility of life, past and present, on other planets in our solar system.

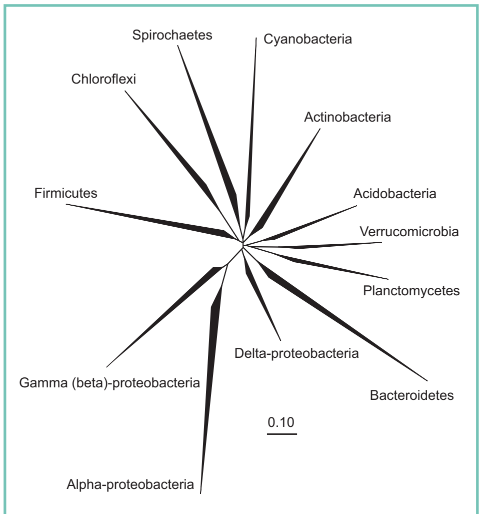

Figure 1. Phylogenetic tree showing phyla detected in deep-subsurface goldmine fracture-water samples by clone and high-density array analysis

APPROACH

We have obtained fracture water samples from 2–3 km below ground level from multiple gold mines of the Witwatersrand Basin in South Africa. These water samples have been dated by isotopic methods as being 14 Kyr to 20 Myr old, with an energy system that appears to be sulfate-and hydrogen-based. To identify the microbial populations in the fracture water, we used a standard clone-library approach and a novel high-density microarray approach. Metagenomic analyses of extracted DNA were carried out at the DOE Joint Genome Institute and at Berkeley Lab to investigate the physiological capabilities of the dominant organism(s).

ACCOMPLISHMENTS

A deep-branching clade of nearly identical *Desulfotomaculum*-like, 16S rDNA sequences (>99% homology) was identified as the dominant microorganism in the planktonic phase of the deepest (2–3 km depth), most saline fracture water. The closest cultured relative is *Desulfotomaculum kuznetsovii* (90% similarity). Further sequences detected by clone library and high-density-array analysis included relatively abundant Firmicutes (*Bacillus*) and less abundant Alpha-, Beta-, Gamma and Delta-proteobacteria, *Cyanobacteria, Chloroflexi, Acidobacteria, Bacteroidetes, Actinobacteria, Spirochaetes, Verrucomicrobia* and *Planctomycetes*. For metagenome analyses, 2.3 Mb of DNA was assembled into 53 scaffolds, and 2,140 likely protein coding genes were identified. Preliminary analyses indicate that the dominant *Desulfotomaculum*-like organism (DLO) represents a new family of organisms and is almost certainly a SO_4 reducer, based on its genomic content. The gene repertoire used for SO_4 reduction is largely the same described for other sulfate-reducing bacteria (SRB), including both delta-proteobacteria and the archeon *Archeoglobus fulgidis*. Although it is a gram-positive organism, it shares a surprising number of its closest homologs with a diverse set of SRB, including *Desulfovibrio vulgaris, D. alaskensis* G20, *D. psychrophila*, and *A. fulgidis*. A number of close homologs were also observed to related organisms such as the S-reducing *Geobacter* and *Desulfuromonas* spp., and the SO_4 oxidizing *Chlorobium tepidum*. This dominant DLO most likely has flagellar motility controlled by a relatively small set of methyl-accepting chemotaxis proteins.

SIGNIFICANCE OF FINDINGS

Clone libraries and high-density microarrays allowed us to profile both the dominant and nondominant fraction of the microbial community in this sulfate/hydrogen-driven subsurface ecosystem. Through metagenome sequencing, we are able to gain insight into the growth strategy of the dominant *Desulfotomaculum*-like organism, which appears capable of sulfate reduction linked to either hydrogen or formate oxidation, in addition to chemotactic motility. This data will enable a better understanding of adaptation and survival of microorganisms in isolated deep-subsurface systems.

RELATED PUBLICATIONS

Brockman, F., D. Moser, T. Gihring, D. Culley, E. Brodie, G. Andersen, T.C. Hazen, P. Richardson, L. Pratt, and T.C. Onstott, Inferred bioenergetics of an uncultured bacterium common in fracture fluids of South African deep mines. NASA Astrobiology Institute (submitted abstract), Biennial Meeting, Boulder, Colorado, 2005.

ACKNOWLEDGMENTS

Funding for this project was provided by the NASA Astrobiology Institute (NAI) and the NSF LExEn (Life in Extreme Environments) Program. We thank the team members of the Witwatersrand Microbiology Project and geologists at the Mponeng Mine for their assistance in field sampling and coordination of logistic supply.

CHARACTERIZATION OF FRACTURE ZONATION USING SEISMIC DATA AND MCMC METHODS

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RESEARCH OBJECTIVES

Our goal in this study is to delineate fracture zonation in order to understand field-scale bioremediation experiments carried out at the DOE Field Research Center (FRC) in Oak Ridge, Tennessee, using crosswell seismic-travel-time and borehole-flowmeter-test data. Our focus is on the development of an effective joint inversion model for combining the crosswell geophysical data with the borehole measurements to characterize fracture zonation in high resolution. The joint inversion approach was developed to minimize problems sometimes encountered with sequential two-step hydrogeophysical estimation approaches, which entails initial inversion of the geophysical dataset, followed by the use of that dataset to estimate hydrogeological parameters. In this example, seismic anisotropy and discrepancies between the borehole and crosshole measurement support scales prohibited the meaningful use of the conventional two-step hydrogeophysical inversion approach for identifying the spatial distribution of fracture zones.

APPROACH

We developed a true joint inversion approach for estimating fracture zonation by combining crosswell seismic travel-time and borehole-flowmeter-test data using a Bayesian framework. First, we transferred the continuous values of hydraulic conductivity data, obtained from borehole flowmeter test data, into indicator values (1 = high conductivity, 0 = low conductivity), using the median of hydraulic conductivity as the cutoff value. Then, we created a Bayesian model to estimate the probability of being within the high-conductivity fracture zone at each pixel in space, by conditioning crosswell travel-time data and indicator values of borehole flowmeter test data. In the model, the prior probability of being within the high conductivity zone at each pixel was determined using an indicator kriging method. The likelihood function, which links seismic slowness (the inverse of seismic velocity) and hydraulic conductivity, was determined from cross-correlation analysis. Finally, we used Markov chain Monte Carlo methods (MCMC) to draw many samples of the indicator values at each pixel in space. Through statistical analysis of those samples, we obtained the probability of being within the high-conductivity fracture zone at each pixel.

ACCOMPLISHMENTS

Figure 1 illustrates the probability of being within the high-conductivity fracture zone along three vertical cross sections at the FRC site, obtained using the developed joint stochastic inversion approach. The cross sections illustrate the value of the developed procedure for such investigations: in some cases, interpolation of borehole data might be sufficient for representing the connectivity of the fracture zone, while in other cases, such interpolation could lead to incorrect assumptions about fracture zone connectivity. Our fracture zonation estimates were corroborated using other types of information, such as field tracer breakthrough data and bioremediation results.

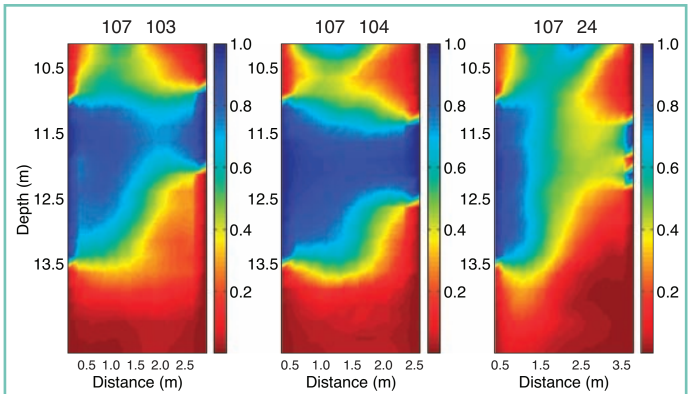

Figure 1. Estimated probability of being within the high-conductivity fracture zones along three transects at the DOE NABIR Field Research Center in Oak Ridge, Tennessee. The blue color represents high probability, the red color represents low probability.

SIGNIFICANCE OF FINDINGS

We have found that the developed stochastic MCMC model is effective for combining seismic travel-time and borehole-flowmeter-test data. This is one of the first examples of true joint inversion of geophysical and hydrological information for hydrogeologic investigations. The study also illustrates the value of using joint inversion methods for subsurface characterization, especially in "challenging" subsurface environments (such as the FRC), where conventional hydrogeophysical approaches fail to provide quantitative property estimates.

RELATED PUBLICATIONS

Chen, J., and S. Hubbard, Development of a joint hydrogeophysical inversion approach and application to a fractured aquifer. Water Resour. Res. (in preparation), 2005.

ACKNOWLEDGMENTS

This work was supported by the Director, Office of Science, Office of Biological and Environmental Research, Environmental Remediation Sciences Division, Natural and Accelerated Bioremediation Research (NABIR) Program, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

UNRAVELING THE HISTORY OF URANIUM CONTAMINATION TO THE VADOSE ZONE IN THE T-WMA, HANFORD SITE, WASHINGTON

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RESEARCH OBJECTIVES

For decades, the Hanford, Washington, site was used to process nuclear fuels for the production of plutonium. These past industrial-scale activities resulted in local contamination of the vadose zone and groundwater. An important consideration in the process of cleanup is the source and fate of such contaminants as uranium (U). Our previous work in the B-BX-BY waste management area demonstrated the power of high-precision U isotopic measurements in providing practical constraints on the sources of U contamination, as well as on the behavior and transport of U in the vadose zone. Here we extend this work to two cores that sampled contaminated vadose zone sediments in the T and TX waste management areas (WMAs), laying the groundwork to trace the source of recently recognized ^{99}Tc groundwater contamination in the vicinity of the T-WMA.

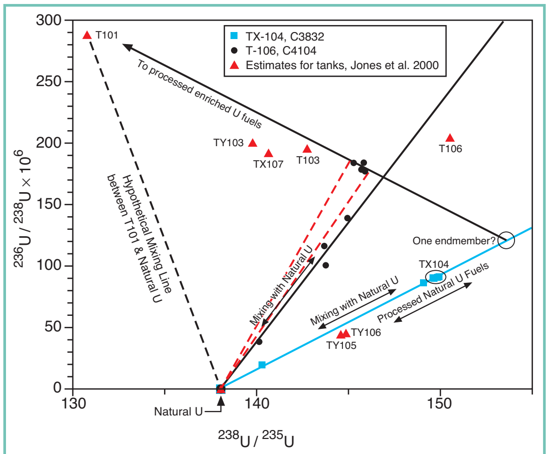

Figure 1. Plot of ^{238}U/^{235}U vs. ^{236}U/^{238}U × 10^6. Black circles are analyses of U from pore water from core C4104 near T-106, blue squares are analyses of pore water from core C3832, near TX-104. Also shown are estimates of tank waste compositions for the T-TX-TY WMAs at their declared leak dates.

APPROACH

The isotopic composition of natural U contrasts with the variable isotopic composition of U from processed fuel rods. This variation can be used as a tracer and fingerprint of contamination. Samples were analyzed for U isotopic composition from two cores (C4104 and C3832) near two single-shelled tanks, T-106 and TX-104, respectively. Uranium from pore waters in the sediment samples were separated and analyzed for isotopic composition by a multiple-collector inductively-coupled-plasma-source mass spectrometer (MC-ICPMS). The results of the isotopic analyses were evaluated and compared to each other to identify sources and the extent of mixing with background U. The isotopic data were also compared to models of the U fuel processing history to provide some temporal constraints.

ACCOMPLISHMENTS

The U isotopic analyses for Core C4104 near Tank T-106, known to have leaked over a six-week period in 1973, indicate that the contaminant U introduced to the vadose zone at this location had a component of processed enriched U fuel, along with a component of processed natural U fuel (Figure 1), at most about 20% and at least 80%, respectively. Moreover, consideration of ^{234}U/^{238}U data (not shown here) constrains the composition of the processed natural U fuel end member. The proportion of processed enriched U decreases down core, until the U isotopic compositions fall along a mixing array with background natural U. In the case of the U isotopic analyses for Core C3832 near Tank TX-104, most of the data form a tight cluster on the array, representing natural U fuels.

SIGNIFICANCE OF FINDINGS

For vadose zone U contamination associated with tanks BX-102 (BX WMA, 200 East Area) and TX-104 (TX WMA, 200 West Area), we found that the contaminant U isotopic composition was highly homogeneous. In contrast, the core near T-106 shows a more complex history, involving mixtures of natural U fuel and enriched fuel, with variable proportions apparently through time. It appears either that contamination was decanted from a tank stratified in U isotopic composition, or that the vadose zone contamination represents mixing with near-contemporaneous contamination from nearby Tank T-103. In either case, the down-core mixing array with background U suggests that the earlier contamination had a lower proportion of processed enriched fuel, providing a basis for modeling the mobility of U in the vadose zone. The data for C4104 also establishes the U isotopic signatures of the waste leak associated with T-106, providing an important part of the isotopic context needed to evaluate the source of ^{99}Tc groundwater contamination in the vicinity of the T-WMA.

RELATED PUBLICATION

Christensen, J.N., P.E. Dresel, M.E. Conrad, K. Maher, and D.J. DePaolo, Identifying the sources of subsurface contamination at the Hanford Site in Washington, using high-precision uranium isotopic measurements. Environ. Sci. Technol., 38 (12), 3330–3337, 2004. Berkeley Lab Report LBNL-54979.

ACKNOWLEDGMENTS

This work was supported by the Assistant Secretary of the Office of Environmental Management, Office of Science and Technology, Environmental Management Science Program, of the U.S. Department of Energy, under Contract No. DE-AC03-76SF00098 to LBNL and Contract No. DE-AC06-76RL01830 to PNNL.

Tracing Sources of Uranium to the Hanford Reach of the Columbia River

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RESEARCH OBJECTIVES

As part of the World War II-era Manhattan Project, the Hanford Site was established along the Columbia River to take advantage of local hydroelectric power and ample water supply for cooling of a series of nuclear reactors. Decades of nuclear-related activities have left significant local contamination (e.g., nitrate, uranium (U), tritium, Cr^{6+}, ^{99}Tc) in the vadose zone and groundwater within the site. Some of this contamination has reached the Columbia River, and there remains the potential for further contaminant migration to the river. We collected and analyzed samples of Columbia River water for U and strontium (Sr) isotopes in coordination with the ongoing sampling and monitoring of the river. The U and Sr isotopic data allow us to evaluate sources of U (e.g., natural background, Hanford related, agricultural runoff) and their relative contributions to the river's U budget. The data also provide constraints on the flux and sources of contaminant U from the Hanford Site to the river.

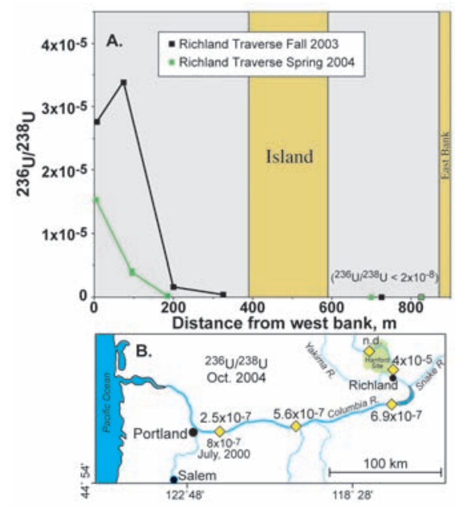

Figure 1. (a) River sampling traverse across the Columbia River at the Richland pump house, about 5 km downstream of the Hanford Site 300 Area. Shown are $^{236}U/^{238}U$ data for sampling in fall 2003 and spring 2004. The sampling traverse is divided by an island. (b) Map showing locations of the Hanford Site and downstream sampling sites along the Columbia River, with indicated $^{236}U/^{238}U$. N.D. = not detected.

APPROACH

We analyzed three sample traverses across the Columbia, one near the Vernita Bridge, upstream from Hanford Site contamination, a second just downriver of the 300 Area, and a third about 5 km downstream of the Hanford Site, adjacent to the town of Richland. Additional samples were collected up to 350 km downstream. Sampling was conducted in fall 2003, spring 2004, and fall 2004. Filtered (0.45 micron) water samples were analyzed for U isotopic composition (including ^{236}U, one marker of spent U fuel) and U concentration, as well as $^{87}Sr/^{86}Sr$ and Sr concentration. Uranium isotopes were measured by multiple-collector-inductively-coupled-plasma source mass spectrometer (MC-ICPMS), and Sr isotopes by thermal ionization mass spectrometry (TIMS).

ACCOMPLISHMENTS

The samples from the upstream traverse had no detectible ^{236}U ($^{236}U/^{238}U < 2 \times 10^{-8}$), natural $^{238}U/^{235}U$, uniform $^{234}U/^{238}U$, $^{87}Sr/^{86}Sr$, or U and Sr concentrations. In contrast, the downstream traverses showed variation in all of these parameters. As an example, $^{236}U/^{238}U$ data are shown in Figure 1a for the fall 2003 and spring 2004 Richland traverses. The $^{236}U/^{238}U$ highlight a plume of U contamination coming from the Hanford Site. Correlation of $^{236}U/^{238}U$ with $^{238}U/^{235}U$ is consistent with a component of 2nd cycle enriched U fuels. For the river water sample with the highest $^{236}U/^{238}U$, about 30% of the total U comes from Hanford contamination. For the eastern portion of the traverse, no detectable ^{236}U was found (Figure 1a). However, $^{234}U/^{238}U$ and $^{87}Sr/^{86}Sr$ (not shown) indicate significant contributions from agricultural return canals about 6 km upstream of the traverse. Measurable $^{236}U/^{238}U$, though small, was observed in all the downstream samples (Figure 1b), and together with $^{238}U/^{235}U$ was consistent with a Hanford Site source.

SIGNIFICANCE OF FINDINGS

The isotopic compositions of the U-contaminated river samples are consistent with a U-contaminated groundwater source in the 300 Area, where fuel elements (both 2nd cycle enriched and natural U) were fabricated. In fact, particular 300 Area groundwater and seep samples are matched as sources, pointing to particular points along the shore.

Both U concentrations and $^{236}U/^{238}U$ decrease downstream (Figure 1b), indicating not only progressive dilution of the Hanford U signature, but also suggesting that at the same time U was being lost from solution (in this case, the <0.45 mm fraction) to particulates. Comparing fall 2003 and spring 2004 Richland traverses, we have found that the flux and apparent source of Hanford U changes with season, with higher relative flux in the fall.

RELATED PUBLICATION

Christensen, J.N., P.E. Dresel, M.E. Conrad, G.W. Patton, and D.J. DePaolo, Tracing and apportioning sources of uranium to the Hanford reach of the Columbia River using uranium isotopes. Eos Trans. AGU, 85(47), F858, 2004.

ACKNOWLEDGMENTS

This work was supported by the Assistant Secretary of the Office of Environmental Management, Office of Science and Technology, Environmental Management Science Program, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098 to LBNL and Contract No. DE-AC06-76RL01830 to PNNL.

Nanotechnology and Environmental Policy: Analysis of Funding and Outcomes

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RESEARCH OBJECTIVES

Nanotechnology and nanoscience can lead to great advances in many fields, and the potential revolutions they may bring to medicine, energy systems, and information technology have been widely publicized. However, the potential societal and environmental implications are a growing concern. There have been a few recent reports that initiate the discussion of environmental impact, but these are not yet comprehensive or conclusive. This work analyzes the National Nanotechnology Initiative (NNI) funding related to the environmental impact of nanotechnology and the research outcomes in this area.

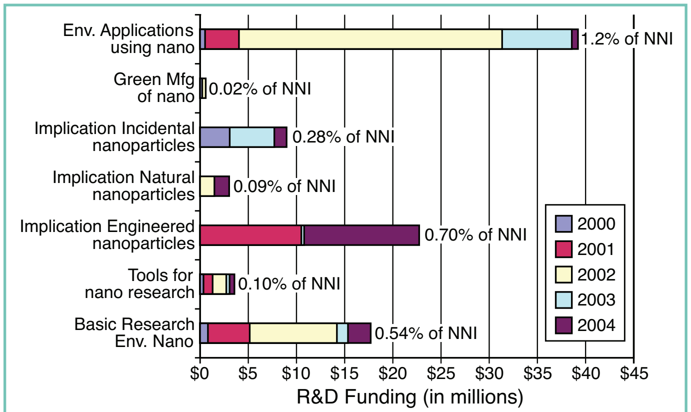

Figure 1. Estimated NNI environmental research, 2000–2004. These preliminary results include an overestimate of funding in the "implication of engineered nanoparticles" area.

APPROACH

We use an ecosystem approach to define "environmental impact" such that it encompasses positive, negative, and neutral effects on soil, air, water, plants, animals (including humans), and other organisms. We obtained data from several sources: agency websites, interviews with agency representatives, and the NNI website. All funding data were assigned a coded category, shown in Figure 1. To code funding data, we reviewed the available title, abstract, and progress-report data on individual grants, and categorized these grants by research topic. In some instances, the principal investigators of the grants were contacted to identify the distribution of the awards by category.

ACCOMPLISHMENTS

We analyzed the NNI funding data for nanotechnology and the environment from 2000–2004 cumulatively, as well as by year and agency, as shown in Figure 1. Preliminary estimates indicate that funding to date in all environmental nanotechnology studies is only 2.9% of the $3.26 billion of federal grant money coordinated by the NNI in this period. This environmental nanotechnology funding is heavily weighted towards "environmental applications of nanotechnology," or the positive environmental uses of nanotechnology. "Implication of engineered nanoparticles" received a little over half of the overall funding for positive environmental applications, but includes a significant funding increase in 2004.

We also analyzed the published research on environmental implications of nanotechnology. Toxicity of nanosized aerosol particles and carbon fullerenes has been discussed in the literature for a number of years prior to the establishment of the NNI. Recent studies on nanotechnology impact are limited, and focus on the toxicity of nanoparticles such as cadmium selenide, carbon nanotubes, and fullerenes. Limited studies on nanoparticle exposure and transport of nanoparticles also have been published. Many of the studies cite enhanced toxicity or anomalous behavior at the nanoscale, reinforcing the need to investigate the unique impact of nanosized particles on all aspects of the environment.

SIGNIFICANCE OF FINDINGS

Funding priorities for environmental nanotechnology do not yet appear to have stabilized. This situation is likely to be the result of shifting "top-down" considerations of the environmental issues related to nanotechnology by government officials. It is important, however, to consider the influence that "bottom-up" factors have had on this result, because the ultimate distribution of funds according to topic area must reflect the proposed research of scientists applying for NNI environmental grants. The limited published studies on the overall environmental impact of nanotechnology demonstrate that there are many interesting and vital studies to be conducted in this area.

RELATED PUBLICATIONS

Banfield, J.F., and H. Zhang, Nanoparticles in the environment. In: Nanoparticles and The Environment: Reviews in Mineralogy and Geochemistry, J.F. Banfield and A. Navrotsky, eds., 44, 1–59, 2001.

Taylor, M.R., E.S. Rubin, and D.A. Hounshell, Stimulating environmental technological innovation: Government actions and SO_2 control technology. Technological Forecasting and Social Change, November 2004.

ACKNOWLEDGMENTS

This work was supported by a President's Postdoctoral Fellowship, provided by the Executive Director, President's Postdoctoral Fellowship Program, Department of Academic Advancement, Division of Academic Affairs, of the University of California Office of the President. This work was also partially supported by the Assistant Director, Directorate of Geosciences, Division of Earth Sciences, Nanoscale Interdisciplinary Research Team Program, of the National Science Foundation under Contract No. EAR0123967.

Berkeley Lab
http://www-esd.lbl.gov/

DOE Environmental Management International Projects Highlights

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RESEARCH OBJECTIVES

Berkeley Lab provides technical assistance to the U.S. Department of Energy (DOE) Environmental Management (EM) Office in conducting projects with Russia, Ukraine, and Argentina. The overall objective of these EM international projects is to use foreign sites as analogs for DOE contaminated sites, to:

- Improve the capability of DOE's conceptual and numerical models for predicting radionuclide transport through unsaturated and saturated subsurface media.
- Predict the future environmental and human impact of radioactive contaminant releases.
- Assist DOE sites in designing effective remediation technologies and long-term stewardship programs.

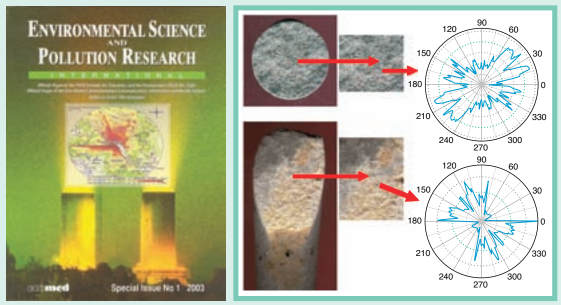

Figure 1. *Environmental Science and Pollution Research Journal* cover page (left), and photographs of basalt core and RIMAPS results (right)

APPROACH AND ACCOMPLISHMENTS

In 2004–2005, Berkeley Lab assisted DOE-EM in designing site characterization, radionuclide transport modeling, and selecting soil and groundwater remediation technologies at the Russian Research Center "Kurchatov Institute" (RRC KI). Information about the radionuclides leaking from individual waste disposal facilities, and the field observations of radionuclide distribution in groundwater at the RRC KI site, can be used to validate the source-term assessment approaches being used at nuclear waste disposal sites in the U.S.

The results from investigations conducted within the Chernobyl Exclusion Zone, established after the Chernobyl Nuclear Power Plant Accident of 1986, suggest several important potential areas of collaboration between DOE and the International Radioecology Laboratory (Slavutych, Ukraine). These collaborative possibilities include the development and testing of models for radionuclide migration through the chain of "fallout-soils-plants-animals-humans"; radionuclide transport in soils and groundwater; bioremediation, natural attenuation, and deactivation of radioactive contaminants; colloidal transport; nondestructive determination of Pu, Am, and Sr in hot particles, water, and soil samples (using alpha-, beta-, and gamma spectrometers as well as a fluid scintillation spectrometer); and resuspension of radionuclides as a result of wind and soil erosion.

The projects with Argentina consisted of two components: (1) numerical modeling and characterization of groundwater flow and contaminant transport at the Ezeiza Atomic Center, and recommendations for source containment and groundwater remediation; and (2) investigations of the physics of liquid flow and transport through partially saturated fractures. Within the scope of the second task, Argentine scientists developed a new technique, rotated imaging with a maximum average power spectrum (RIMAPS). This technique is used for the digital characterization of a fracture-surface topography, which is then applied to assess the main flow paths along the fracture surface. The RIMAPS technique was tested at Comisíon Nacional de Energía Atómica using (a) digital images of the fracture pattern and flow paths from fracture model flow experiments conducted at Berkeley Lab and (b) a fractured basalt core collected at the Box Canyon site in Idaho (Figure 1). The application of this technique will allow researchers to predict the water flow parameters from the analysis of fracture surfaces.

SIGNIFICANCE OF FINDINGS

It was determined that the international sites chosen could be used as analogs for some DOE contaminated sites. DOE would likely benefit from access to long-term and detailed monitoring of contaminant transport in the variety of contaminant, climatic, and geological settings provided by these analogous sites. These projects will allow DOE researchers, engineers, and managers to use international scientific resources to test and build confidence in DOE's fate and contaminant transport models and remediation technologies, which will allow DOE to reduce costs, improve remedy and mitigation reliability, and increase the effectiveness of remediation technologies during long-term stewardship of DOE sites.

RELEVANT PUBLICATION

Fuentes, N.O., and B.A. Faybishenko, RIMAPS and variogram characterization of water flow paths on a fractured surface. In: Proceedings of the Second International Symposium on Dynamics of Fluids in Fractured Rock, pp. 120–123, Berkeley, CA, 2004.

ACKNOWLEDGMENTS

This work is supported by the Assistant Secretary of the Office of Environmental Management, Office of Science and Technology, Environmental Management Science Program, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. The above-mentioned projects are managed by the DOE Environmental Management Office (project manager Kurt Gerdes), and the Institute for International Cooperative Environmental Research of Florida State University (Associate Director John Moerlius). DOE supported publication of a special issue of the *International Journal Environmental Science and Pollution Research* devoted to Chernobyl problems (Figure 1).

Biological Treatment of Irrigation Drainage for Selenium Removal

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RESEARCH OBJECTIVES

Much of the subsurface agricultural drainage in the western San Joaquin Valley (SJV) is contaminated with selenate (50–1,200 mg/L as Se) and nitrate (20–120 mg/L as N), in addition to high total dissolved solids (TDS) and boron. This water is currently either discharged to sloughs that drain into the San Joaquin River and then to the San Joaquin Delta, or it is evaporated in terminal ponds. These means of disposal are problematic because selenium is a teratogen that bioaccumulates in the aquatic food web, and because nitrate contaminates groundwater supplies and promotes eutrophication of surface waters. Nitrate also interferes with the reduction and removal of selenate, SeO_4^{-2}, the most abundant form of selenium found in western SJV drainage. Our objective is to develop reliable and economical treatment methods to remove these contaminants.

APPROACH

From Pilot Plant to Prototype Facility

We have developed the algal-bacterial selenium removal (ABSR) process to remove nitrate and selenium from irrigation drainage. A 75 m^3/day pilot-scale ABSR Facility has been used to study the mechanisms and rates of selenium and nitrate removal. Based on the success of the pilot facility, a 10-fold scale-up intermediate-scale ABSR Facility may be implemented. In the ABSR process, subsurface drainage is dosed with a carbonaceous energy source for bacteria (usually animal feed-grade molasses) and then injected into a baffled and covered anoxic reduction pond. In the reduction pond, bacteria denitrify nitrate and reduce selenate to selenite, elemental selenium, and bacterial-associated organic selenium. Much of the reduced selenium settles in the pond. Settled bacterial biomass in the reduction pond undergoes anaerobic decomposition, so the volume of solid residues increases very slowly. Removal of the selenium-containing solids should not be required for many years, possibly decades. The effluent water from the reduction pond is coagulated, clarified, and filtered to remove suspended bacteria and selenium-containing suspended solids.

ACCOMPLISHMENTS

Selenium Removal

Over two years, the pilot-scale ABSR Facility at the Panoche Drainage District has removed 95% of the influent nitrate-nitrogen load and 80% of the influent soluble selenium load. Addition of physical-chemical treatment processes, including dissolved air flotation and filtration processes to remove particulate selenium, has increased total selenium removal to 87%. Dozens of bacterial species have been isolated from the ABSR Facility and identified by 16S rRNA sequencing, including the prevalent *Acinetobacter Johnson*II/genospecies 7, *Pseudomonas mendocina*, and *Xanthomonas maltophilia*. Pure cultures of several of these bacteria have been proven to reduce selenite in the laboratory.

Brine Treatment

Planned "zero discharge" drainage management in the SJV will create brines that require treatment. The high salt concentration of brines may inhibit bacterial selenium reduction, thereby increasing the cost of irrigation drainage treatment. We have found that denitrification and selenate reduction are unaffected by NaCl concentrations augmented up to 22 g/L. Above 22 g/L, however, reduction is substantially inhibited. This information is important in the planning and design of proposed integrated-reverse-osmosis and biological-drainage treatment processes.

SIGNIFICANCE OF FINDINGS

With the ABSR Facility at the Panoche Drainage District, we have demonstrated a promising, cost-effective process that will be used in planning full-scale facilities to remove nitrate and selenium from irrigation drainage.

RELATED PUBLICATIONS

Green, F.B., T.J. Lundquist, N.W.T. Quinn, M.A. Zárate, I.X. Zubieta, and W.J. Oswald, Selenium and nitrate removal from agricultural drainage using the AIWPS® Technology. Water Science and Technology, 48 (2): 299–305, 2003. Berkeley Lab Report LBNL-55205.

Sudame A., S. Lee, H. Lee, T. Lundquist, P. Muller, K. Hida, H. Ng, P. F. Strom, and T. Leighton, Selenite-reducing bacteria of Panoche Algal Bacterial Selenium Removal (ABSR) Facility, California. In: Proceedings of 34th Mid-Atlantic Industrial and Hazardous Waste Conference, 159–172, September 2002.

Quinn, N.W.T., T.J. Lundquist, F.B. Green, M.A. Zárate, W.J. Oswald, and T.J. Leighton, Algal-bacterial treatment facility removes selenium from drainage water, California Agriculture, 54 (6), 50–56, 2000. Berkeley Lab Report LBNL-50318.

ACKNOWLEDGMENTS

We are grateful to the U.S. Bureau of Reclamation, the CALFED Bay-Delta Program, Department of Water Resources, and the Panoche Drainage District for their support.

Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at the Hanford 100H Area

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RESEARCH OBJECTIVES

Field investigations have been performed to assess the potential for immobilizing and detoxifying chromium-contaminated groundwater, using lactate-stimulated bioreduction of Cr(VI) to Cr(III), at the Hanford Site's 100H Area.

APPROACH AND METHODS

Lactate (Hydrogen Release Compound—HRC™) injection into chromium-contaminated groundwater is expected to cause indirect or direct bioreduction of chromate, Cr(VI), and precipitation of insoluble species of Cr(III). At Hanford 100H, two new wells were completed using a newly developed assembly with inflatable (argon gas) rubber packers, groundwater samplers, and an inner geophysical access tube. Pre-HRC injection and post-HRC injection geophysical (seismic and radar) cross-borehole measurements were performed (see a summary by Hubbard et al., 2005 [this volume]). Forty pounds of ^{13}C-labeled HRC were injected into Well 699-96-45 (44–50 ft within the Hanford formation) on August 3, 2004, immediately followed by pumping (which continued until August 30) from the Monitoring Well 699-96-44.

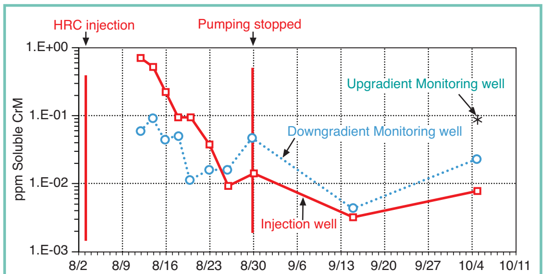

Figure 1. Changes of Cr(VI) concentration in injection and monitoring well resulting from HRC groundwater biostimulation

Groundwater analyses included: Acridine orange direct counts and molecular analyses—PLFA, 16S GeneChip, clone library, qPCR, bromide (tracer added to the injection well), chloride and phosphate (added to HRC), acetate (byproduct of HRC microbial metabolism), nitrate and sulfate (present in background groundwater), Cr(VI), total Cr, and Fe(II), total Fe, carbon, nitrate, and oxygen isotopic compositions.

ACCOMPLISHMENTS

Groundwater biostimulation caused microbial cell counts to increase from a background of ~10^5 cells g^{-1} to reach a maximum of 2×10^7 cells g^{-1} 13 to 17 days after the injection. This maximum lasted for 2 months and then decreased to values even less than those under pre-HRC-injection conditions. Biostimulation also generated highly reducing conditions: DO dropped from 8.2 to 0 mg/L, redox potential from 240 to -130 mV, and pH from 8.9 to 6.5. After pumping stopped and the system returned to natural regional groundwater flow, DO, redox, and pH began to recover to background values. PLFA and direct counts both indicated similar biomass changes. Carbon isotope ratios of DIC decreased, but remained for 6 months above background in Well 699-96-44 and within the injection interval in Well 699-96-45—until December 2004. The $\delta^{13}C$ ratios in dissolved inorganic carbon confirmed microbial metabolism of HRC. Geophysical investigations show that HRC products (such as lactic acids) injected into groundwater can be detected using radar and seismic survey, and that even small variations in hydrogeological heterogeneity may influence the distribution of the amendment and its products.

Hydrogen sulfide production was first observed after about 20 days post-injection, which corresponds with the enrichment of a *Desulfovibrio* species (sulfate reducer) identified using 16S rDNA microarray and monitored by direct fluorescent antibodies. DO and nitrate began to return to background concentrations two months after HRC injection, despite groundwater bacteria densities remaining high (>10^7 cells/mL).

As a result of groundwater biostimulation, Cr(VI) concentrations in the monitoring and pumping wells decreased below drinking water minimum-contaminant limits and remained below upgradient concentrations, even after 6 months (Figure 1), when redox conditions and microbial densities had returned to background levels.

SIGNIFICANCE OF FINDINGS

Microbial, geophysical, and geochemical analyses of groundwater, coupled with stable isotope monitoring, allowed for accurate tracking of microbial processes during this field treatability study, and confirmed that Cr(VI) was successfully removed from groundwater at a contaminated site using HRC as an electron donor and carbon source.

PROJECT WEBSITE

http://www-esd.lbl.gov/ERT/hanford100h/index.html

RELATED PUBLICATIONS

Hubbard, S., K. Williams, J. Peterson, J. Chen, B. Faybishenko, and T. Hazen, Geophysical monitoring of HRC distribution in groundwater during a Cr(VI) bioreduction experiment at the Hanford 100H site. This Volume, 2005.

ACKNOWLEDGMENTS

This work was supported by the Director, Office of Science, Office of Biological and Environmental Research, Environmental Remediation Sciences Division, Natural and Accelerated Bioremediation Research (NABIR) Program, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. The work was conducted jointly by Berkeley Lab, Pacific Northwest National Laboratory, and Regenesis, Ltd.

VIMSS: Large-Scale Biomass Production of Obligate Anaerobes for Simultaneous Transcriptomics, Proteomics, Metabolomics, and Lipidomics Analysis

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RESEARCH OBJECTIVES

The Virtual Institute for Microbial Stress and Survival (VIMSS) at Lawrence Berkeley National Laboratory (Berkeley Lab) seeks to identify stress-response pathways of *Desulfovibrio vulgaris* induced by various environmental factors, by combining multiple simultaneous analyses in an effort to conceptualize these pathways. The Applied Environmental Microbiology core at Berkeley Lab is responsible for producing large quantities of *D. vulgaris* biomass for different research laboratories, to accomodate simultaneous analyses on cells with the same growth condition and stress level. Biomass production at Berkeley Lab involves the production of nonstressed and stressed *D. vulgaris* culture at various time points during the growth phase, which is a four-month process with two major stages. During Stage 1, Berkeley Lab determines the stressor and the dosage, as well as designing and carrying out a multi-time point experiment for Oak Ridge National Laboratory's transcriptome analysis. During Stage 2, Berkeley Lab will produce 12 to 30 liters of biomass for all the VIMSS laboratories, based on the results of transcriptomics. Berkeley Lab is also responsible for all QA/QC verifications, all sample shipments, the uploading of data, and analysis for all experiments.

APPROACH

To rapidly determine induced-stress-response pathways in anaerobic microorganisms, we need to produce biomass for simultaneous analyses, using the latest techniques in transcriptomics, proteomics, metabolomics, and lipidomics. To accomplish this, batch cultures of 30 liters of stressed versus nonstressed *D. vulgaris*, as biological replicates in triplicates, are needed to ensure that all the analyses will be performed on cells of the same condition. Various technical improvements and adaptations were made for the large-scale production and distribution of biomass exposed to a variety of stressors, such as oxygen, salt, nitrate, nitrite, and temperature. Because of the rapidly changing nature of DNA and the short half-life of mRNA, *D. vulgaris* cultures needed to be immediately cooled to 5°C during biomass sampling. As a result, a fast sample-cooling system was developed to chill biomass from 30°C to 5°C in less than 30 seconds. Because of the concomitant analysis by several laboratories, rigorous quality control measures were used to ensure the quality and sterility of biomass from each time point in a production run (e.g., direct cell counts, optical density, pH, plate streaks, phospholipid fatty acid [PLFA] analysis, and protein assays). In addition, advanced Fourier Transform Infrared (FTIR) spectromicroscopy profiling was used to study gross bimolecular changes and to determine optimal sampling times. QA/QC procedures were developed and documented to track every step in production, from experiment inception to final analyses, including all chemicals, procedures, and technicians. Data are immediately uploaded to a database shared by all investigators (http://vimss.lbl.gov)

ACCOMPLISHMENTS

Over a 20-month period from September 2003 to May 2005, the Applied Environmental Microbiology core at Berkeley Lab conducted over 40 large-scale *D. vulgaris* biomass production experiments and countless small-scale experiments. During this time, the group developed various techniques and made numerous improvements to VIMSS biomass production, such as in media composition, anaerobic sampling, and biomass harvesting. Direct filtration and tangential flow filtration were studied as viable cell harvesting alternatives to centrifugation. However, studies showed that centrifuging *D. vulgaris* cells was the best option in terms of time, cost, efficiency, and quality control. The consistency of growth determined by the comparison data allows a set biomass production schedule and sampling time, which minimize variability between experiments.

SIGNIFICANCE OF FINDINGS

- Biomass production of batch cultures in biological replicates demonstrated a reliable and carefully controlled method to inoculate, grow, stress, and sample *D. vulgaris* cultures.
- QA/QC verifications at every stage of biomass production insure maximum reproducibility between biomass production experiments.
- Centrifugation and the fast chilling system appropriately prepared replicate samples simultaneously for transcriptomics, proteomics, metabolomics, and lipidomics processing.
- The large-scale biomass production of *Desulfovibrio vulgaris* for stress response studies can be used as a model for the large-scale production of other obligate anaerobes in the future.

RELATED WEB SITE

http://vimss.lbl.gov

ACKNOWLEDGMENTS

This work was part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program: Genomes To Life (GTL) through Contract No. DE-AC03-76SF00098 between Berkeley Lab and the U. S. Department of Energy.

Geophysical Monitoring of Amendment Distribution and Reactivity During a Cr(VI) Bioreduction Experiment at the Hanford 100-H Site

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RESEARCH OBJECTIVES

The efficacy of *in situ* contaminated-groundwater remediation, using the injection of chemical or biological amendments, depends on the ability to control their distribution within contaminated heterogeneous media. However, understanding how amendments are distributed in natural subsurface systems is difficult to ascertain using conventional (wellbore) characterization techniques, which often sample only a very localized area. In this study, we explore the use of time-lapse geophysical data for imaging amendment distribution as a function of time and heterogeneity. The geophysical research was performed as part of a Cr(VI) bioreduction experiment at the Hanford 100H Site in Washington, where Hydrogen Release Compound (HRC™; a slow-release polylactate amendment, is being used to reduce Cr(VI) into insoluble Cr(III) complexes. Hazen, et al. (2005; this volume) provide more details on the Cr(VI) bioreduction study.

APPROACH

These estimates were determined using seismic and radar crosshole data sets (collected before the injection experiment) with wellbore flowmeter data in a discriminant analysis technique. In August 2004, HRC was injected through the injection well into a Hanford sand/gravel saturated aquifer. Pumping was initiated simultaneously to "pull" the HRC products towards the downgradient monitoring well. Cross-borehole field seismic and radar tomographic data were collected during and subsequent to amendment injection, and were then compared with those data acquired prior to the injection. Geophysical data were also compared with the results of analytical analyses of water samples collected from both wells. Because the HRC and its byproducts are likely to change the electrical conductivity of porous solution, radar tomographic amplitude and velocity data were used to estimate the electrical conductivity changes between the injection and pumping wells.

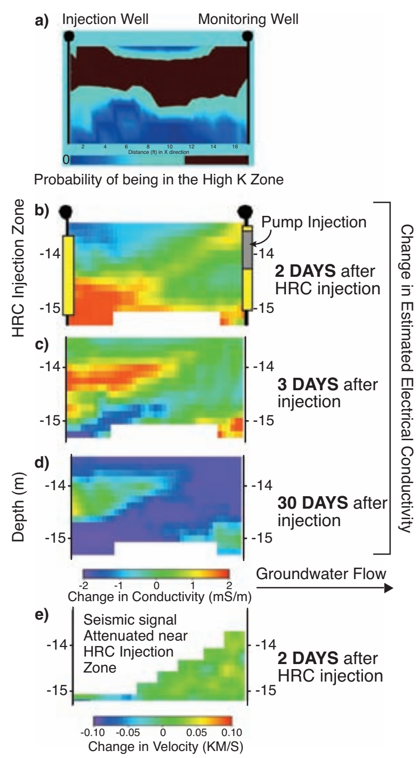

Figure 1. Estimates obtained using geophysical tomographic data: (a) probability of being in a high conductivity zone where black indicates higher hydraulic conductivity; changes in electrical conductivity, (b) 2 days after HRC injection, (c) 3 days after injection, and (d) 30 days after injection; (d) changes in seismic velocity 2 days after HRC injection.

ACCOMPLISHMENTS

Figure 1a depicts the zonation of hydraulic conductivity of the Hanford formation. Figure 1b indicates the initial increase in the estimated electrical conductivity near the base of the injection interval, which was likely caused by the release of the lactic acid upon hydration of the HRC in groundwater. Figure 1c demonstrates that after 3 days, the effect of pumping from the monitoring well pulled the lactic acid into the higher hydraulic conductivity zone. After 30 days, while the electrical conductivity remained practically the same near the injection well, it decreased (blue areas in Figure 1d) downgradient from the injection well. We hypothesize that this change is likely associated with formation of precipitates. HRC injection caused the seismic response to completely attenuate immediately after the HRC injection (Figure 1e). The field-scale geophysical responses to the HRC injection agree with the results of observations conducted during a series of laboratory controlled HRC-injection and geophysical monitoring experiments.

SIGNIFICANCE OF FINDINGS

The high-resolution, field-scale, cross-borehole geophysical (seismic and radar) measurements hold significant potential for imaging the spatial distribution of lactate-based amendments in heterogeneous sediments, and may be useful for detecting chemical transformations (such as precipitates) of metals. These results also indicate the importance of heterogeneity in controlling amendment distribution. Continuation of this research is necessary to further explore the concept of using geophysical techniques for assessing the remediation efficacy of contaminated sites.

RELATED WEBSITE

http://esd.lbl.gov/ERT/hanford100h/

ACKNOWLEDGMENTS

This work was supported by the NABIR Program, Office of Science, Environmental Remediation Sciences Division of DOE's BER Program under Contract No. DE-AC03-76SF00098. All computations were carried out at the Center for Computational Seismology supported by DOE's Basic Energy Science Program.

Groundwater Flow Monitoring and Plume Evolution

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RESEARCH OBJECTIVES

In the mid-1990s, investigators observed transgradient expansion of the groundwater plume of halogenated, aliphatic volatile organic compounds (VOCs) at Operational Unit 1 (OU-1) of the former Fort Ord Army Base in Monterey, California. Additionally, the plume was found to extend considerably farther in a downgradient direction than determined in the initial characterization performed during the late 1980s. A possible second contaminant release site was posited to explain this latter observation. The objective of this project was to use advanced flow and analyte monitoring instrumentation, efficient data management and analysis, and numerical modeling to understand the evolution of the OU-1 plume.

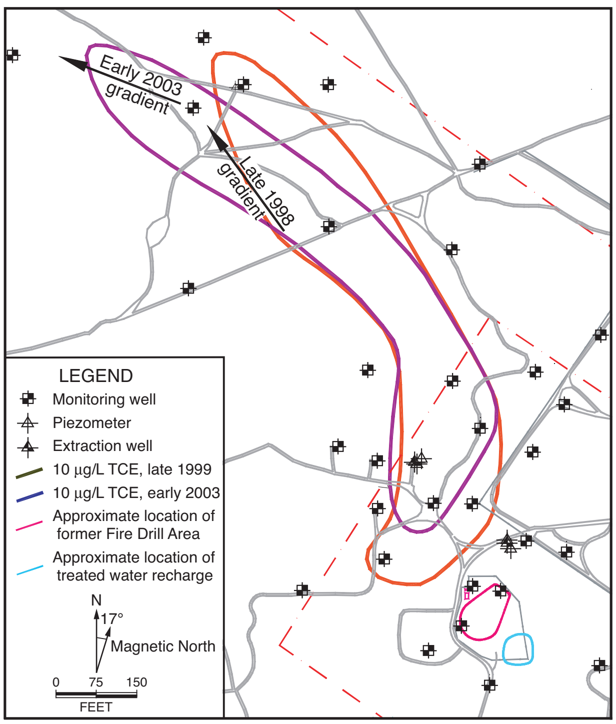

Figure 1. Gradient rotation and extent of trichloroethylene (TCE) plume greater than 10 µg/L after historically intense precipitation in early 1998 at OU-1, Former Fort Ord Army Base

APPROACH

Well-log, water-level, and chemistry data were provided by the groundwater monitoring contractor for OU 1, MacTec Engineering and Consulting, Inc. Treatment system totalizer data were provided by the remedial contractor, AHTNA Government Services, Inc. To improve analysis efficiency, and therefore insight, water-level and chemistry data were loaded into the environmental information management system software (EIMS) GIS\Key (produced by GIS Solutions, Inc.), which integrates data management with graphing and mapping. Precipitation data for the area were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Data Center and incorporated into the analysis. We installed five Hydrotechnics heat-based *in situ* flow sensors to measure well-scale groundwater flow velocities (magnitude and direction). In addition, we carried out short-duration pump tests to measure hydraulic conductivity.

The data set aggregated from the above activities was analyzed to determine the hydrogeologic structure, precipitation recharge rates, hydraulic gradient, contaminant concentrations, porosity, and hydraulic conductivity distribution, orientation, and scaling. A conceptual model of the relationship between these parameters was tested through numerical modeling.

ACCOMPLISHMENTS

The hydraulic conductivity of the aquifer materials was found to be remarkably uniform across scales, orientations, and locations. The numerical modeling demonstrated that the entire OU-1 plume evolved from one contaminant release site. Concentration trend analysis indicated that treated water recharge occurred within the plume area and therefore caused transgradient expansion of the plume. Water-level analysis revealed that gradients in the distal portion of the plume rotated counterclockwise upwards of 30º in response to historically intense precipitation in early 1998 (see Figure 1). Concentration trend analysis further revealed advection of the distal portion of the plume oblique to the plume axis, as expected because of the plume rotation.

SIGNIFICANCE OF FINDING

The rotation of the plume at OU 1 has profound implications. One implication is the need for a significantly expanded well field to monitor and remediate the plume over time as it rotates. These results demonstrate the value of an integrated approach to plume monitoring that includes flow sensors for local groundwater velocity measurement, increased efficiency of data analysis provided by an EIMS, and numerical simulation.

RELATED PUBLICATIONS

Jordan, P.D., C.M. Oldenburg, and G.W. Su, Analysis of aquifer response, groundwater flow, and plume evolution at Site OU 1, former Fort Ord, California. Berkeley Lab Report LBNL-57251, February 2005.

ACKNOWLEDGMENTS

This work was supported by University of California Santa Cruz through the U.S. Army Construction Engineering Research Laboratories under Contract No. DACA42-02-2-0056, and by the U.S. Department of Energy under Contract DE-AC03-76-SF00098.

Joint Inversion of Ground-Penetrating Radar and Hydrological Measurements

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RESEARCH OBJECTIVES

Ground-penetrating radar (GPR) measurements are not directly related to soil hydraulic parameters (e.g., permeability and the parameters of the capillary pressure and relative permeability functions). However, GPR measurements are highly sensitive to fluid distribution (and to transients therein) and are thus potentially useful for inferring soil hydraulic parameters in the vadose zone, especially when combined with additional data types. The objective of this study is to develop a method that jointly uses cross-borehole GPR measurements and hydrological measurements to provide quantitative estimates of field-scale soil hydraulic parameters.

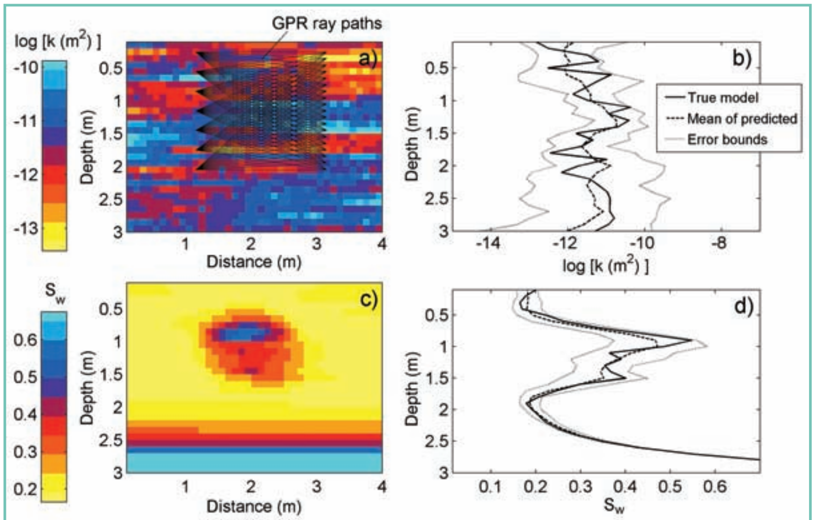

Figure 1. A heterogeneous permeability model (a) is used to simulate a water injection experiment. Joint inversion of synthetic GPR and neutron probe data allow for accurate estimates of permeability (b), which can be used to predict flow phenomena, such as the water saturation profile at a given time (c–d). Vertical cross sections through the two-dimensional models of (a) and (c) are shown in (b) and (d), respectively.

APPROACH

How a hydrological system responds to external stimuli, such as the injection of water, is influenced by the soil hydraulic parameters and their variations in space. Corresponding GPR measurements of the same system also depend on the soil hydraulic functions—although indirectly—since the soil hydraulic functions influence the water distribution, which in turn influences the GPR measurements. Our approach allows for the estimation of soil hydraulic parameters through the coupled simulation (and inversion) of multiple-offset cross-borehole GPR travel times and hydrological measurements collected during transient flow experiments. Joint inversion proceeds by perturbing the unknown hydraulic parameters—which alters the simulated water distributions and subsequent geophysical observations—until a good match is achieved between the simulated and measured (geophysical and hydrological) observations.

ACCOMPLISHMENTS

Within the context of water injection experiments, we tested the approach with synthetic examples and also applied it to field data. The synthetic examples show that while realistic errors in the petrophysical function (the function that relates soil porosity and water saturation to the effective dielectric constant) cause substantial errors in the soil hydraulic parameter estimates, simultaneously estimating petrophysical parameters and soil hydraulic parameters allows for these errors to be minimized. Additionally, inaccuracy in the GPR simulator can cause systematic error (bias) in the simulated travel times, making necessary the simultaneous estimation of a correction parameter. After demonstrating the usefulness of the method with synthetic examples (Figure 1), we applied it in a three-dimensional field setting to field data (GPR and neutron probe data) collected during an infiltration experiment at the U.S. Department of Energy (DOE) Hanford site in Washington. We find that inclusion of GPR data in the inversion procedure provides hydrological models that predict water-content distributions better than models obtained using neutron probe data alone.

SIGNIFICANCE OF FINDINGS

GPR and other geophysical methods offer high resolution and minimally invasive information that has traditionally been difficult to relate to hydrological properties. Our joint inversion approach provides a way to incorporate geophysical data into hydrological investigations in a meaningful and quantitative way. The flexible framework we have developed should prove useful for inclusion of additional geophysical data types, such as from seismic and electrical methods.

RELATED PUBLICATIONS

Kowalsky, M.B., S. Finsterle, and Y. Rubin, Estimating flow parameter distributions using ground-penetrating radar and hydrological measurements during transient flow in the vadose zone. Advances in Water Resources, 27 (6), 583–599, 2004. Berkeley Lab Report LBNL-53786.

Kowalsky, M.B., S. Finsterle, J. Peterson, S. Hubbard, Y. Rubin, E. Majer, A. Ward, and G. Gee, Estimation of field-scale soil hydraulic and dielectric parameters through joint inversion of GPR and hydrological data. Water Resources Research (in press), 2005. Berkeley Lab Report LBNL-57560.

ACKNOWLEDGMENTS

This work was supported by Laboratory Directed Research and Development (LDRD) funding from Berkeley Lab, provided by the Director, Office of Science, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

A Multisensor System for Detection and Characterization of UXO

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RESEARCH OBJECTIVES

The objective of this field demonstration project is to show that a multisensor active electromagnetic (AEM) system can be built to detect and extract essential information about a metallic object in the ground, so as to discriminate unexploded ordnance (UXO)-like bodies from non-UXO scrap. Further, we hope to demonstrate that the system can perform target characterization from a single position of the sensor platform above a target. This system will equal or exceed the detection capabilities of existing systems, but with the important advantage of being able to quantitatively determine size and principal polarizabilities of the target. The cart-based system is designed to detect and characterize UXO in the 20 mm to 150 mm size range.

APPROACH

Drawing on the experience gained in the completion of Strategic Environmental Research and Development Program (SERDP) Project UX-1225 (Detection and Classification of Buried Metallic Objects), we are building an optimally designed field prototype system. The system employs two orthogonal vertical loop transmitters and a pair of horizontal loop transmitters vertically spaced 0.7 m apart. Eight vertical field detectors are deployed in the plane of each of the horizontal loops and are arranged to measure the offset vertical gradients of the fields. The system employs a bipolar half-sine pulse-train current waveform, and the detectors are dB/dt induction coils designed to minimize the transient response of the primary field pulse. The sensor coil pairs are located on symmetry lines through the center of the system, so that they detect identical primary fields (for all three transmitters) during the on-time of the pulse. These coil pairs are wired in opposition to produce a null output. Secondary fields from the target have a large gradient that is easily measured in the differenced output. Field prototype sensors are critically damped and resonant at about 20 kHz, and resonant frequency allows a 270 Hz waveform repetition rate and a duty factor of about 20%. The location and orientation of the three principal polarizabilities of a target can be recovered from a single position of the transmitter-receiver system. Further characterization of the target is obtained from the broadband response.

ACCOMPLISHMENTS

We have developed a field-prototype active EM system that can extract from the measurements the best possible estimates of the location, size, shape, and metal content of a buried metallic object, in the presence of an interfering response from the ground and/or non-UXO metallic objects. The prototype system has been tested in the laboratory with very encouraging results. This project received the SERDP Project of the Year award in the UXO Field at the Partners in Environmental Technology Symposium in Washington on December 2, 2004. A rigorous field test is under way and will be followed by a demonstration survey at an Environmental Security Technology Certification Program (ESTCP) standard test site.

RELATED PUBLICATIONS

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ACKNOWLEDGMENTS

This work was supported by the Office of Management, Budget, and Evaluation, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098, and the U.S. Department of Defense under Strategic Environmental Research and Development Program Project No. UX-1225 and Contract No. W74RDV30452524.

Remote-Sensing Techniques for Assessing Impacts of Wetland Real-Time Water Quality Management on Wetland Seasonal Habitat

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RESEARCH OBJECTIVES

The U.S. Bureau of Reclamation has recently announced its intention to require wetlands in the western U.S. to develop best management practices (BMP's). The formulation of BMP's requires detailed knowledge of current practices, together with accurate water balances and estimates of evapotranspiration, to allow assessment of water-use efficiency. The Bureau of Reclamation has entered into a collaboration with Berkeley Lab to develop remote - sensing tools to improve estimates of evapotranspiration and to assess habitat value based on quantitative estimates of moist soil plants. Recent advances in available image data and processing techniques have increased the scope for success in discriminating between moist soil plants found in managed wetlands in California's Central Valley. Models such as SEBAL and METRIC use thermal infrared data, in combination with visible and near-infrared data, to estimate evapotranspiration. Vegetation mapping can help extrapolate these estimates to the entire wetland resource area. This study explored the development of an accurate, consistent, and efficient methodology for mapping land cover and vegetation through remote-sensing technology.

APPROACH

Multispectral satellite imagery was used to map vegetation and other land cover in Central Valley wetlands. The imagery displayed reflected light in blue, green, red, and near-infrared wavelengths. High-resolution image data enabled mapping of small and irregularly shaped vegetation communities. An integrated GPS/field computer permitted rapid collection of consistent ground-truth data. The protocol for describing vegetation communities was based on the California Native Plant Society's Rapid Assessment Protocol, and a comprehensive field guide was developed for identification of plant species. Image data were processed using the software packages ERDAS Imagine and Definiens eCognition. Spectral signatures were developed using field data to guide the selection of representative pixels. A maximum likelihood algorithm was used to classify each pixel according to its statistical similarity to defined classes.

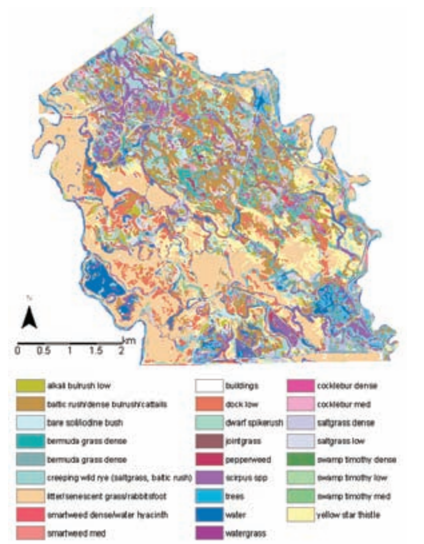

Figure 1. Map of land-cover classes derived from satellite image data using statistically based image processing. Image classification is an efficient process, using a few thousand known pixels to classify an entire image comprised of tens of millions of pixels.

ACCOMPLISHMENTS

A land-cover map was produced for an area of Central Valley wetlands covering 160 km^2. Figure 1 shows a map of land-cover classes represented in the San Luis National Wildlife Refuge. Most important vegetation communities, including those dominated by alkali bulrush, baltic rush, cocklebur, and swamp timothy, were represented with greater than 75% accuracy. Other classes, such as bermuda grass, smartweed, and watergrass, were classified with lesser accuracy. Overall, the remote-sensing methodology mapped a large area, using minimal field data, with a high degree of accuracy.

SIGNIFICANCE OF FINDINGS

Central Valley wetlands are significant water users and compete with agriculture for an adequate water supply. Effluent impacts water quality in the San Joaquin River, and real-time management of drainage may be incorporated into BMP's for these wetlands. Management decisions regarding water should be evaluated with respect to habitat health. A remote-sensing mapping methodology can provide an accurate and consistent means to track changes in habitat. Accurate land-cover maps also provide the basis for water-needs analyses through quantitative assessment of evapotranspiration from open water, bare soil, and vegetation communities.

RELATED PUBLICATION

Quinn, N.W.T, W.M. Hanna, J.S.Hanlon, J.R. Burns, C.M. Taylor, D. Marciochi, S. Lower, V. Woodruff, D. Wright, and T. Poole, Real-time water quality management in the grassland water district. Berkeley Lab Report LBNL-56825, 2004.

ACKNOWLEDGMENTS

This work was supported by CALFED Ecosystems Restoration Program administered by the U.S. Bureau of Reclamation under the U.S. Department of Interior Interagency Agreement N.3-AA-20-1097, through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Fluid Logging Experiments to Determine Depth Distribution of Salts Beneath Seasonally Flooded Wetlands

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RESEARCH OBJECTIVES

Groundwater conjunctive use in California is being promoted by the State and Federal water resource agencies as a means of alleviating over-allocated water supply, especially in the western San Joaquin Valley. The U.S. Bureau of Reclamation, having recently undertaken a study of water banking in the aquifers that underlie seasonally flooded wetlands, has partnered with Berkeley Lab to assess the potential impact of long-term pumping strategies on the quality of the water produced by these wells. Tsang and Doughty (2003) have shown how the flowing-fluid-electric-conductivity (FEC) logging method can be used to yield a profile of water quality along the length of the screened interval in the well. This current research extends this analysis in a very practical way, allowing the technique to be deployed in some nonideal situations, in particular at sites where the motor and well pump could not be removed to access the well casing.

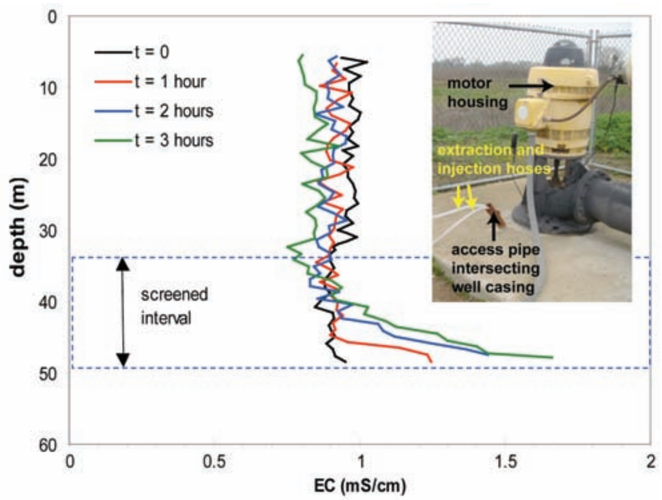

Figure 1. Electrical conductivity profiles measured over time in a well in the San Joaquin Valley as water is extracted at a constant rate of 0.7 m^3/hr. Photograph of a well with limited access is shown in the upper right-hand corner.

APPROACH

FEC logging has been conducted in wells around the wetlands in the San Joaquin Valley. This technique involves replacing the wellbore water with deionized or low-salinity water while the wellbore water is simultaneously extracted. After the wellbore water is replaced by low-salinity water, the change in the electrical conductivity (EC) profile in the well is recorded over time as the water is extracted at a constant rate (Figure 1). The FEC logging technique had previously been performed only in vertical wells with diameters typically ranging between 5 to 15 cm. The wells at our study site had very limited access through a 3.8 cm diameter pipe that intersected the wellbore at a 45º angle (Figure 1). We modified the FEC logging technique such that this method could be used in these wells.

ACCOMPLISHMENTS

The typical EC probe used to log vertical wells has a diameter of 3.8 cm and a length of 1.8 m; such a probe could not fit into the access pipe intersecting our study wells. Instead, we successfully deployed a small EC probe manufactured by Campbell Scientific up to depths of 90 m. The probe has a cross-sectional dimension of 2.5×1.9 cm and is 8.9 cm long. To inject and extract the water simultaneously through the access pipe, we used small-diameter (1.9 cm) hoses. Because we were limited to using such a small hose, we developed and tested a new technique of injecting water uniformly over the well length by inserting pressure-compensating emitters along the length of the hose. This new technique replaces the more time-consuming, traditional approach of injecting low-salinity water only at the well bottom.

SIGNIFICANCE OF FINDINGS

We successfully conducted FEC logging in wells with limited access and obtained transient measurements of the electrical conductivity with depth (Figure 1). Over time the EC increases faster towards the bottom of the well than at the top of the screened interval, indicating that the formation at the bottom of the screened interval is more permeable or the EC of the water at the bottom of the well is higher. Obtaining data on the depth distribution of salts and identifying high salinity zones in the aquifers in the San Joaquin Valley is critical for evaluating the potential of groundwater for conjunctive water use.

RELATED PUBLICATION

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ACKNOWLEDGMENTS

This work was supported by Laboratory Directed Research and Development (LDRD) funding from Berkeley Lab, provided by the Director, Office of Science, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Impact of Agricultural Non-Point-Source Pollution on Water Quality

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RESEARCH OBJECTIVES

Non-point-source (NPS) water pollution is now recognized as the most significant remaining source of water quality impairment in the United States. NPS pollution is a worldwide problem and impacts drinking water and quality of life in both industrial and nonindustrial societies. NPS pollution, unlike pollution from industrial or sewage treatment plants, comes from diffuse sources that are hard to identify and are poorly understood and characterized. Agriculture is a major source of NPS pollution, but the nature, character, and impact of this pollution is largely unknown.

We are currently conducting an ecosystem-level study on the San Joaquin River in the Central Valley of California, examining how NPS discharge impacts water quality. The primary objective is to understand how nutrient and sediment-laden drainage from agriculture influences primary productivity (algal growth) in the river, and how algal growth produces secondary impacts on water quality and fish habitats in the river and connecting delta ecosystem. We are also examining how changing agricultural practices, referred to as best management practices (BMPs), will influence productivity and water quality in riverine ecosystems.

APPROACH

To understand the interaction between NPS pollution, algae growth rate, and algae biomass carrying capacity, we are measuring a complete mass balance on algae and nutrients over a 110 mile reach of the San Joaquin River. Simultaneous collection of water quality and biological data at 21 sites in the watershed develops an instantaneous "snapshot" or profile of algae and nutrients in the river. Seasonal and diurnal changes in algal productivity are studied, using continuously deployed chlorophyll measuring devices and field experiments in which individual algal blooms are tracked and characterized. Specific tributaries and "hot spots" for algal growth are subject to focused studies to answer basic scientific and engineering questions concerning environmental conditions limiting algal growth. The installation and operation of a network of 51 stations, for the continuous measurement of flow and salt concentration in the main stem of the river and the tributaries, provides the final piece of the puzzle that allows us to develop a complete mass balance on algae and nutrients in this ecosystem.

ACCOMPLISHMENTS

This year is the first of a three-year study. Collection of water quality and biological data has begun. The continuous-flow and water-quality monitoring network is almost complete. Studies of bloom dynamics and individual tributaries are in progress.

SIGNIFICANCE OF FINDINGS

Initial studies have already contributed to our understanding of NPS pollution in the San Joaquin Valley. Studies of algal growth and water quality at previously uncharacterized tributaries have raised new questions concerning our understanding of algal growth dynamics in this river. Such studies have challenged assumptions included in water quality models used by state agencies to manage water quality in this severely impacted water body.

RELATED PUBLICATIONS

Stringfellow, W.T., and N.W.T. Quinn, Discriminating between west-side sources of nutrients and organic carbon contributing to algal growth and oxygen demand in the San Joaquin River. CALFED Bay-Delta Program, Sacramento, California; Berkeley Lab Report LBNL-51166, 2002.

ACKNOWLEDGMENTS

This research is supported by funding from the California Bay-Delta Authority Ecosystem Restoration and Drinking Water Quality Programs, the State Water Resources Control Board, the Center for Science and Engineering Education, and the University of the Pacific Environmental Engineering Research Program.

Development of an Unsaturated Region Below a Perennial River

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RESEARCH OBJECTIVES

Field observations along the Russian River in Sonoma County, California, indicate that an unsaturated region exists below the streambed near two adjacent collector wells located along the riverbank. Understanding the conditions that give rise to unsaturated flow below the streambed is critical for improving and optimizing riverbank well-pumping operations. A three-dimensional model was developed using TOUGH2 to investigate the conditions under which an unsaturated region develops below a perennial river when the collector wells were pumping.

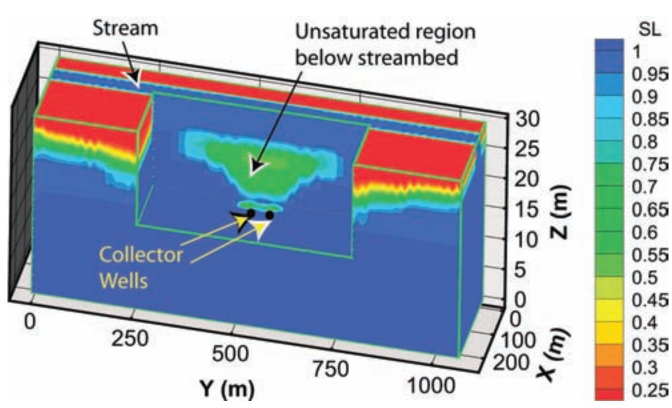

Figure 1. Simulated water saturation (SL) below the streambed after the two collector wells had pumped continuously for seven days at 1,600 m^3/hr. The aquifer permeability is 2.4×10^{-10} m^2 and the streambed permeability is 7.4×10^{-13} m^2.

APPROACH

The numerical model was based on the region near two collector wells in the Russian River Bank Filtration Facility. These wells consist of nine perforated pipes that are projected horizontally into the aquifer at a depth of approximately 20 m below the land surface. A grid was developed that was highly refined near the wells, so that the individual pipes could be represented. The two collector wells each pumped continuously at a rate of 1,600 m^3/hr.

ACCOMPLISHMENTS

The aquifer below the streambed remained saturated when the aquifer and streambed permeability were the same (both 2.4×10^{-10} m^2), and when the streambed permeability was one order of magnitude smaller than the aquifer permeability. When the streambed permeability was 2.4×10^{-12} m^2, which was two orders of magnitude smaller than the aquifer permeability, an unsaturated region developed below the streambed that was approximately 25 m wide, 130 m long, and 3 m deep. When the streambed permeability was 7.4×10^{-13} m^2, which was 2.5 orders of magnitude less than the aquifer permeability, a large unsaturated region formed below the river that extended across the entire river width (60 m), was over 350 m long, and up to 10 m deep. Under these conditions, the simulated unsaturated region that developed near the streambed after seven days of continuous pumping is shown in Figure 1.

SIGNIFICANCE OF FINDINGS

As the permeability of the streambed decreased relative to the aquifer permeability, the extent of the unsaturated region below the streambed increased. The results of the numerical simulations have important implications for well operation. During the summer and fall months, when the inflatable dam is raised at the Russian River Bank Filtration Facility and the streambed permeability decreases over time, well operation may have to be altered if the permeability decreases to a value such that a large unsaturated region forms below the streambed.

RELATED PUBLICATION

Su, G.W., J. Jasperse, D. Seymour, and J. Constantz, Estimation of hydraulic conductivity in an alluvial system using temperatures. Ground Water, 42(6), 890–901, 2004. Berkeley Lab Report LBNL-53167.

ACKNOWLEDGEMENTS

This work was supported by the Sonoma County Water Agency (SCWA), through U.S. Department of Energy Contract No. DE-AC03-76SF00098.

Interpretation of Groundwater Velocities from Heat-Based Flow Sensors

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RESEARCH OBJECTIVES

Heterogeneities in formation properties around an *in situ* heat-based flow sensor may lead to incorrect interpretations of groundwater flow velocities. The flow sensor operates by constant heating of a 0.75 m long, 5 cm diameter cylindrical probe, which contains 30 thermistors in contact with the formation. The temperature evolution at each thermistor is inverted to obtain an estimate of the groundwater flow velocity vector, based on the assumption that the formation is homogeneous. Analysis of data from three heat-based flow sensors installed in a shallow sand aquifer at the Former Fort Ord Army Base near Monterey, California, suggested a strong and unexpected component of downward flow. Three-dimensional TOUGH2 simulations were conducted to investigate how differences in the thermal conductivity and permeability around the instrument may lead to inaccurate indications of downward flow velocities.

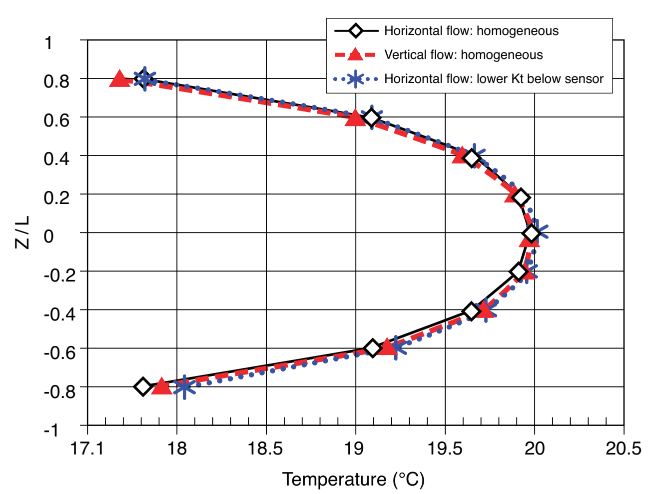

Figure 1. Simulated temperature profiles along the sensor for horizontal and vertical flow in a homogeneous formation, and for horizontal flow when K_t below the sensor is lower than K_t along the sensor

APPROACH

Conformal mapping was used to generate a discretization highly refined near the cylindrical instrument body to use for simulating flow and heat transport near the sensor. A 17-layer model was used in the 3-D simulations, with the heated portion of the flow sensor represented by the nine center layers. Horizontal flow was imposed across the domain in all the simulations, except for one where vertical flow was imposed. Heterogeneity was assumed only in the vertical direction, resulting in a "layer-cake" type stratigraphy.

ACCOMPLISHMENTS

Simulated temperature profiles on the downstream side of the flow sensor are plotted as a function of depth in Figure 1. In a homogeneous formation, the temperature profile is symmetric for horizontal flow, while higher temperatures are observed along the bottom half of the sensor relative to the top half for vertical (downward) flow. When the thermal conductivity (K_t) of the formation is lower below the sensor compared to the thermal conductivity along the length of the sensor (2.1 versus 1.8 W/m°C), the temperature profile becomes shifted in such a manner that it could be interpreted as resulting from downward flow, even though flow is actually horizontal. A decrease in the permeability towards the bottom of the sensor relative to the top can also result in a temperature profile that could be interpreted as having a downward flow component, although the effect tends to be smaller.

SIGNIFICANCE OF FINDINGS

The simulations demonstrate that the temperatures recorded by heat-based flow sensors are sensitive to differences in the thermal and hydraulic properties of the formation. Under conditions of strictly horizontal flow, the temperature profile along the length of the sensor can be perturbed by changes in the thermal conductivity and permeability, such that analysis of the data assuming homogeneous formation properties could result in interpreting the temperature shift as the result of downward flow.

RELATED PUBLICATIONS

Su, G.W., B.M. Freifeld, C.M. Oldenburg, P.D. Jordan, and P.F. Daley, Simulation of *in situ* permeable flow sensors for measuring groundwater velocity. Ground Water (in review), 2005. Berkeley Lab Report LBNL-57084.

Jordan, P.D., C.M. Oldenburg, and G.W. Su, Analysis of aquifer response, groundwater flow, and plume evolution at Site OU1, Former Fort Ord, California. Berkeley Lab Report LBNL-57251, 2005.

ACKNOWLEDGMENTS

This work was supported by U.C. Santa Cruz (UCSC) through the U.S. Army Construction Engineering Research Laboratories (US ACERL), and by the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Use of Biomarker Sequences for the Identification and Phylogenic Analysis of Filamentous Fungi Isolated from Extreme Environments

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RESEARCH OBJECTIVES

Fungi play complex and diverse roles in ecosystems. Their importance to biotechnology and bioprospecting is enhanced by the vast diversity of extant fungi. The search for novel capabilities and useful new genetic traits most often targets microorganisms that live under extreme environmental conditions, such as high temperature, extreme pH, salinity, and radiation. Scientists at Berkeley Lab's Center for Environmental Biotechnology have been bioprospecting for many years at contaminated sites and closed military bases, in deserts and forests in the USA, in Lake Baikal sediments and at geothermal and hydrothermal sites on the Kamchatka peninsula in Russia, and at the failed nuclear power plant and within the surrounding 30 km "Exclusion Zone" in Chernobyl, Ukraine. Their work focuses on filamentous fungi that often possess the ability to produce unique secondary metabolites with potential commercial value. These natural products are advantageous to the organism in their respective environment as chemical defense against predators, pathogens, or competitors. Identification of these microorganisms provides a better understanding of their ecological function. The overall goal of this project is the taxonomic characterization of thousands of fungal cultures in a way that is amenable to a much wider range of laboratories.

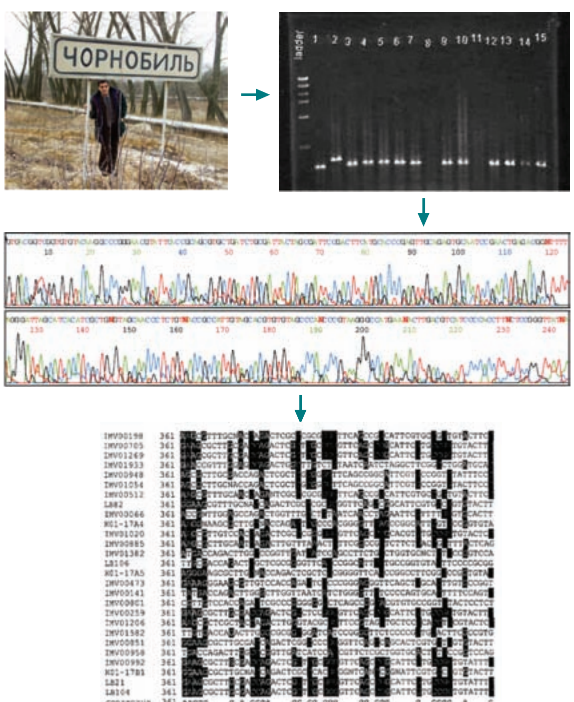

Figure 1. Flow diagram of the sequence-based identification (from environmental sample to the consensus of aligned sequences)

APPROACH

Procedures traditionally used for fungal identification rely on colony-and cell morphology and other distinctive biochemical reactions. Recently, molecular-level protocols have been increasingly used for fungal identification. Here, we applied a polymerase chain reaction (PCR) combined with amplicon sequencing and comparative sequence analysis of biomarker genes. DNA sequencing was done at the University of California-Berkeley DNA Sequencing Facility. Raw sequences were edited and aligned using online multiple sequence aligner subroutines. Aligned sequences were further analyzed for consensus and finally queried against the National Center for Biotechnology Information database for species determination (Figure 1).

ACCOMPLISHMENTS

The main objective of this study was to test an alternative method of fungal identification. Though the number of strains/species/genera included in this study was biased by the extreme environmental habitats, most of over 100 species tested so far were identified correctly, in agreement between classical and molecular-level identification. More definite results will be available when the sequence-based identification of the over 2,000 filamentous fungi isolated from extreme environmental samples is completed.

SIGNIFICANCE OF FINDINGS

Taxonomic identification and detailed characterization of extremophillic microorganisms provides a greater understanding of their diversity, unique metabolism, and ecological function. Here, fungal organisms were characterized successfully by comparing classical techniques and sequence-based phylogenetic differentiation. Because we consider filamentous fungi major sources of novel biotechnology and biomedical applications, a future goal is to design an Affymetrix-type microarray to aid in better understanding of fungal phylogenetic relatedness and diversity.

RELATED PUBLICATIONS

Torok, T., N. Zhdanova, M. Kuchuk, G. Dahlbacka, Amaku, G. Andersen, and J. Hunter-Cevera, Characterization of filamentous fungi isolated from extreme environments. Proceedings of the Fifth International Conference on Extremophiles ("Extremophiles 2004"), p. 86, Cambridge, Maryland, September 19–23, 2004.

ACKNOWLEDGMENTS

We wish to express our deepest appreciation for the support we have received from the U.S. Department of Energy Office of Science and the National Science Foundation. The DOE-supported Initiatives for Proliferation Prevention (IPP) program, and the DOE-NSF jointly sponsored Faculty and Student Training (FaST) program, made progress possible. Special thanks are due to friends and peers.

Enhanced Bioremediation of Contaminated Groundwater at Berkeley Lab, Using Hydrogen-Release Compound®

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RESEARCH OBJECTIVES

The U.S. Department of Energy is actively pursuing innovative, cost-effective methods of remediating contaminated groundwater at its facilities, including Berkeley Lab. The accidental release of solvents used at Berkeley Lab decades ago resulted in contamination of underlying groundwater at several onsite locations. Several groundwater cleanup technologies have been deployed to address the contamination, including conventional groundwater extraction and treatment, soil washing, chemical oxidation, thermally enhanced dual vapor and groundwater extraction, natural attenuation, and enhanced bioremediation. Among these, enhanced bioremediation is a particularly promising, innovative cleanup technology that reduces contaminant levels below regulatory standards in a relatively short period of time.

APPROACH

Indigenous bacteria found in groundwater are known to biodegrade chlorinated solvents, leading to the natural attenuation of groundwater contaminant plumes. Unfortunately, natural attenuation can be a relatively slow process, potentially taking decades to reduce contaminant levels below cleanup requirements. Therefore, supplements that promote bacteria growth can be added to the groundwater to help enhance natural bioremediation and expedite cleanup. This technique is referred to as enhanced bioremediation.

An *in situ* pilot study performed at Berkeley Lab's Building 71B successfully demonstrated rapid biodegradation of chlorinated solvents, within nine months of injecting concentrated glycerol tripolylactate ester into contaminated groundwater. Gycerol tripolylactate (sold under the trade name Hydrogen Release Compound, HRC®) is a commercially available, dense, viscous liquid that is highly soluble in water. Full-scale treatment of the contaminated area, located beneath and downgradient of the building, is currently under way using dilute HRC®. Groundwater is mixed with HRC® and heated in an aboveground bioreactor to stimulate quick bacterial growth, then injected into a drainfield located within the footprint of the building at the source. An existing groundwater pump and treat system, located downgradient from the source, hydraulically controls the plume and supplies water to the drainfield, thus distributing HRC® throughout the contaminated area.

ACCOMPLISHMENTS

Dissolved oxygen and oxidation-reduction potentials rapidly decreased in groundwater within three weeks of HRC® injection. This rapid decrease indicates that reducing conditions favoring anaerobic biodegradation of solvents quickly developed after introducing HRC®. More importantly, total contaminant levels in groundwater decreased throughout the winter months, in contrast to normal trends when concentrations increased because of winter recharge and rising water table conditions (Figure 1).

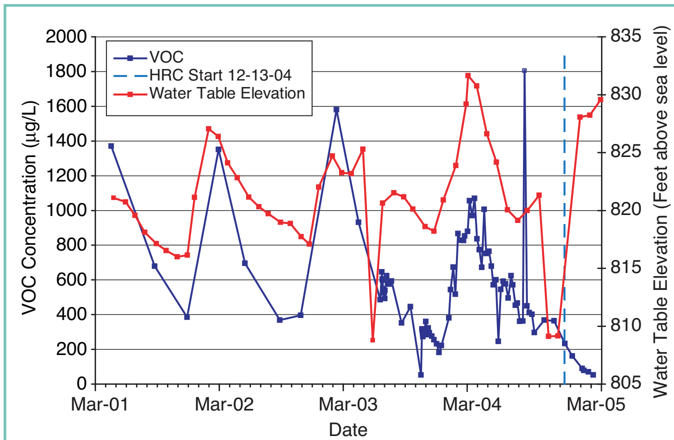

Figure 1. Total volatile-organic compound concentrations increase every year when the water table beneath Building 71B rises because of winter rains. This trend ended after injecting HRC®.

SIGNIFICANCE OF FINDINGS

HRC® is typically applied by direct injection of the concentrated product into the subsurface, using a dense network of direct push or conventionally drilled borings. The size and depth of the plume, which controls the number of borings and amount of HRC® applied, can have a significant impact on remediation costs. Our study shows that HRC® can be successfully incorporated into an existing conventional groundwater extraction system by injecting it into the subsurface in its dilute form. Additional time is needed to study our approach to assess whether biofouling by bacteria will reduce the permeability of the drainfield, production well, or porous material, rendering our approach ineffective. However, few viable alternatives are available that can be used to treat contaminated soils beneath an existing structure, as with HRC®.

ACKNOWLEDGMENTS

This work was performed by the Berkeley Lab's Environmental Restoration Program, which is operated by the University of California for the U.S. Department of Energy under Contract No. DE-AC03-76-SF00098.

Reoxidation of Bioreduced Uranium Under Reducing Conditions

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RESEARCH OBJECTIVES

Interest in the biogeochemical cycle of uranium (U) is growing, especially for remediating contaminated environments. The mobility of U depends strongly on its oxidation state, with U(IV) being much less soluble than U(VI). Therefore, some strategies under development for immobilizing U in contaminated sediments are aimed at promoting precipitation of U(IV) minerals, typically by injecting organic carbon (OC) into sediments to stimulate microbial U(VI) reduction. Previously, observations of OC-stimulated U reduction have only reported fairly short-term results. Our studies are directed at understanding the conditions controlling longer-term stability of bioreduced U.

APPROACH

Columns packed with a U contaminated (206 mg kg^{-1}) sediment obtained from the Natural and Accelerated Bioremediation (NABIR) Program's Field Research Center in Oak Ridge National Laboratory were supplied with sodium lactate (32 mM OC) solutions, and effluent solution chemistry was monitored. U oxidation states were determined nondestructively in soil columns at the Advanced Photon Source, GSECARS beamline, by micro-X-ray absorption spectroscopy (micro-XAS). Fluorescence spectroscopic measurements were conducted to determine the U species in effluents. Microbial communities within sediment columns were characterized by high-density oligonucleotide array analyses and real-time quantitative polymerase chain reaction methods..

ACCOMPLISHMENTS

Our 17-month study showed that bioreduction of U was transient, even under sustained reducing conditions. Micro-XAS showed that U was reduced during the first 80 days, but later (100 to 500 days) reoxidized and solubilized (Figure 1), although a microbial community capable of reducing U(VI) was maintained. OC-stimulated microbial respiration caused increases in (bi)carbonate concentrations and formation of uranyl carbonate complexes, thereby increasing the favorability of U(IV) oxidation. Fluorescence spectroscopy showed that U(VI) in effluents occurred primarily as uranyl tricarbonate and dicalcium uranyl tricarbonate. We hypothesize that kinetic limitations allowed Fe(III) to persist as terminal electron acceptors for U reoxidation.

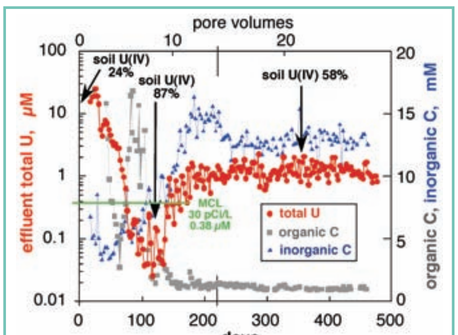

Figure 1. Concentrations of U, OC, and bicarbonate concentrations in effluents from columns supplied with 32 mM OC. Micro- XAS showed that the soil U was initially oxidizing (24% U(IV)), then largely reduced (87% U(IV)), and later reoxidized (58% U(IV)).

SIGNIFICANCE OF FINDINGS

These results show that *in situ* U remediation by OC-based reductive precipitation can be problematic in sediments when uranyl carbonates are stable, and that OC concentrations in remedial solutions need to be carefully considered to minimize carbonate-enhancement of U(VI) solubility. This work also demonstrates the need for long-term experiments to evaluate remediation strategies that rely on transforming actinides and metals to low-solubility products. In considering much longer time scales set by the half-life of ^{238}U, the practicality of reduction-based immobilization strategies in regionally oxidizing sediments needs to be carefully reevaluated.

RELATED PUBLICATIONS

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Wan, J., T.K. Tokunaga, E. Brodie, Z. Wang, Z. Zheng, D. Herman, T.C. Hazen, M.K. Firestone, and S.R. Sutton. Reoxidation of bioreduced uranium under reducing conditions. Environ. Sci. Technol., 39 (in press), 2005. Berkeley Lab Report LBNL-56058.

ACKNOWLEDGMENTS

This project is supported by the Director, Office of Science, Office of Biological and Environmental Research, NABIR Program, and Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences, of the U.S. Department of Energy under Contract No. DE-AC03-76-SF00098. Use of the Advanced Photon Source was supported by the DOE, Office of Science, Basic Energy Sciences. Fluorescence spectroscopic measurements were performed at the Environmental Molecular Sciences Laboratory, sponsored by the Office of Biological and Environmental Research. We thank J. Larsen, D. Joyner, S. Baek, J. Pena, and M. Newville for technical assistance.

Geochemical Evolution of Tank Waste Plumes upon Infiltrating into Sediments

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RESEARCH OBJECTIVES

Leakage of highly saline and alkaline radioactive waste solutions from storage tanks into underlying sediments is a serious problem at the Hanford Site in Washington State. Although it was found from field samples that pH values of the initially highly alkaline (pH 14) waste plumes dramatically decreased (to pH 10–7), understanding of the neutralization process was lacking. Since pH is a master geochemical variable, the behavior of waste plume contaminants, including their speciation, sorption, solubility, precipitation, and transport, can be reliably predicted only when the evolution of the pH profile is understood. This study focuses on the geochemical evolution of major geochemical parameters, including pH, and addresses how pH evolved as the plumes propagated.

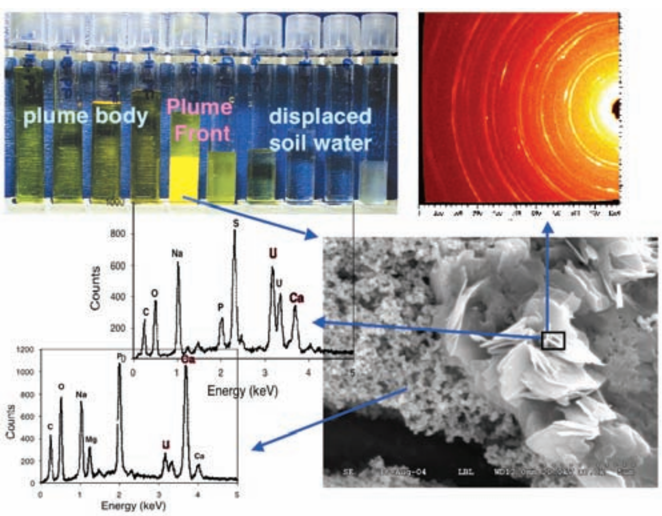

Figure 1. Formation of plume-front colloids from a uranium waste plume. The upper-left photograph shows a profile of extracted pore solution from a U plume and the massive colloid formation at the plume front. The lower-right SEM image shows morphology of the plume front colloids. The EDS analyses (lower-left) indicate U is a major element in these colloid particles. Synchrotron micro-XRD (upper-right) identified the plate-like phase as sodium-uranyl-carbonate.

APPROACH

A plume-profiling method was designed to obtain spatially and temporally resolved direct measurements of plume geochemistry profiles. To simulate the leakage events, we injected synthetic tank waste solutions into Hanford sediment columns at desired temperatures. Experimental conditions were chosen as close to the field conditions as possible, yet simple enough so that causes and effects could be identified. Geochemical and transport processes were studied simultaneously. Finally, we obtained spatially resolved data of geochemical evolution over different waste plume distances and for different times.

ACCOMPLISHMENTS

This study revealed that: (1) a large (i.e., several-units) pH reduction occurred at the plume fronts, which would have a substantial impact on contaminant fate and transport (as demonstrated for uranium); (2) maximum colloid formation occurred at plume fronts (hundreds to thousands times higher than within the plume bodies or during later leaching); and (3) cation exchange of Na^+ replacing Ca^{2+}/Mg^{2+} from the sediments during flow, and precipitation of Ca^{2+}/Mg^{2+}-bearing solids, were identified as being responsible for these plume-front phenomena.

SIGNIFICANCE OF FINDINGS

These plume-front phenomena were not previously revealed by either geochemical modeling or by laboratory batch experiments. This new understanding obtained through our column-based experiments is important for predicting the behavior of contaminants in waste plumes.

RELATED PUBLICATIONS

Wan, J., T.K. Tokunaga, E. Saiz, J.T. Larsen, Z. Zheng, and R.A. Couture, Colloid formation at waste plume fronts. Environ. Sci. Technol., 38, 6066–6073, 2004. Berkeley Lab Report LBNL-56059.

Wan, J., J.T. Larsen, T.K. Tokunaga, and Z. Zheng, pH neutralization and zonation in alkaline-saline tank waste plumes. Environ. Sci. Technol., 38, 1321–1329, 2004. Berkeley Lab Report LBNL-53646.

Wan, J., T.K. Tokunaga, J.T. Larsen, and R.J. Serne, Geochemical evolution of highly alkaline and saline tank waste plumes during seepage through vadose zone sediments. Geochim. Cosmochim. Acta, 68, 491–502, 2004. Berkeley Lab Report LBNL-55733.

ACKNOWLEDGMENTS

This project is supported by the Assistant Secretary of the Office of Environmental Management, Office of Science and Technology, of the U. S. Department of Energy under Contract No. DE-AC03-76-SF00098.

Noninvasive Geophysical Monitoring of Clay-Mineral Transformations During Simulated Iron Reduction

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RESEARCH OBJECTIVES

The importance of iron-bearing clay-sized minerals as a source of bioavailable iron is well documented. During biostimulation, reduction of such accessible ferric compounds by iron-reducing microorganisms can occur rapidly and result in the sequestration of soluble contaminants, such as uranium and chromium in insoluble phases. As the clay-sized fraction is exhausted and more recalcitrant forms of ferric iron are accessed, competition by other microbial strains can result in decreased remediation efficacy. Improved diagnostic methods are needed to elucidate the extent of microbe-induced mineral transformations over the large spatial scales encountered during field experiments.

APPROACH

The complex-resistivity method was used to monitor the effect of iron-reduction at both lab- and field-scales. The lab experiments investigated the effect of both chemical and enzymatic reduction of iron-bearing clays and clay-sized minerals on complex-resistivity signals. The field experiment used an analogous methodology to track the extent of iron reduction that occurred following acetate amendment of a shallow alluvial aquifer near Rifle, Colorado, designed to stimulate microorganisms capable of co-metabolic U(VI)-reduction.

ACCOMPLISHMENTS

Alterations in the physiochemical properties of iron-bearing clays and clay-sized minerals, resulting from both abiotic reduction and microbial respiration, led to decreases in the measured values of complex resistivity at the lab scale (Figure 1b). Reduction of structural iron led to an increase in the layer charge of the clay minerals, which resulted in structural collapse and a decrease in the specific surface area. This decrease in surface area was inferred to be the primary cause of the time-varying complex-resistivity signatures. Similar decreases in the phase response of the complex-resistivity signals were observed during the field biostimulation experiment (Figure 1a). The phase decreases corresponded in both space and time to the onset of microbial iron-reduction and reached a maximum following the cessation of active bioreduction. Mineralogical alterations in the clay-sized fraction of the aquifer sediments were believed to be responsible for the observed geophysical anomalies.

SIGNIFICANCE OF FINDINGS

Noninvasive geophysical monitoring methods have shown sensitivity to the mineralogical changes associated with iron reduction and show promise for monitoring the progress of stimulated subsurface bioremediation at field-relevant scales. Time-varying complex-resistivity anomalies correlated with the exhaustion of bioavailable iron, suggesting an approach for monitoring the sustainability of prolonged iron reduction under stimulated conditions.

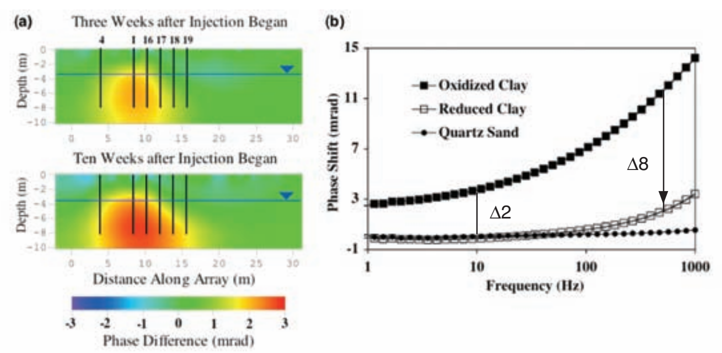

Figure 1. Variations in the complex-resistivity signatures of bioreduced clay minerals resulting from stimulated microbial activity: (a) changes in the phase response measured at the Old Rifle, Colorado, site three and ten weeks after acetate amendment–acetate injection occurred throughout the saturated zone along the location labeled 'I'; and (b) laboratory-derived phase response of oxidized and reduced versions of the same clay mineral as compared to fine quartz sand. The magnitude of the change corresponds to that observed during the field experiments.

RELATED WEBSITE

http://esd.lbl.gov/ERT/sshubbard

ACKNOWLEDGMENTS

This work was supported by the Assistant Secretary of the Office of Environmental Management, Office of Science and Technology, Environmental Management Sciences Program, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. The clay-reduction experiments were carried out at the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by DOE's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory, Washington.

Sourcing Vadose Zone and Groundwater Nitrate Using Nitrate Isotopes

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RESEARCH OBJECTIVES

Nitrate is one of the of the most widespread groundwater contaminants in the United States. There are a number of potential sources for nitrate contamination, including fertilizers, chemical processing, sewage, and elevated natural backgrounds. Analyses of the nitrogen ($\delta^{15}N$) and oxygen ($\delta^{18}O$) isotope ratios of the nitrate represent a powerful tool for distinguishing between different sources. Methods for analyzing the $\delta^{15}N$ and $\delta^{18}O$ values of dissolved nitrate have historically been unwieldy and time-consuming. However, recent advances using denitrifying bacteria to generate N_2O from nitrate have greatly reduced both the preparation time and sample size requirements.

We have streamlined this denitrification technique and are using it for research on the fate and transport of nitrate in the vadose zone. To date, we have applied this technique to tracking nitrate contamination associated with radioactive waste at the Hanford site in south-central Washington and identifying the sources of nitrate in the Exploratory Studies Facility at Yucca Mountain. Highlights of the work at Hanford are presented below.

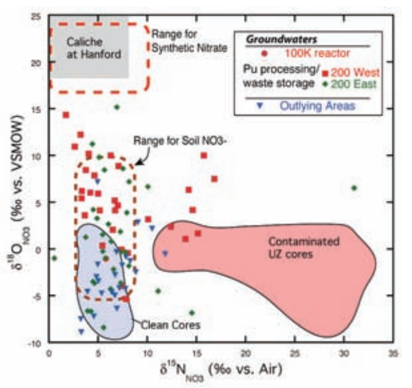

Figure 1. Plot of nitrogen versus oxygen isotope ratios of groundwater nitrate from the Hanford site in south-central Washington. Also shown is the range of values measured for unsaturated zone nitrate from clean and contaminated core samples from Hanford, and the general ranges of isotopic compositions of soil nitrate and synthetic nitric acid.

APPROACH

We modified the existing bacterial technique by increasing initial culture inoculation. We also grow the cultures on the bench top in vials used for our automated headspace sampler and reduced the venting time of the vials before injecting the sample, to decrease sample-processing time. Groundwater samples from the Hanford site were injected directly into inoculated vials. Vadose zone nitrate was extracted from cores by rinsing dried sediment with de-ionized water. The filtered rinse water, assumed to contain the nitrate originally dissolved in the pore fluids, was then injected into the inoculated vials. The vials are then loaded into the headspace sampler and analyzed using an automated trace gas pre-concentration system interfaced to a continuous-flow isotope-ratio mass spectrometer (CF-IRMS).

ACCOMPLISHMENTS

The modifications to the bacterial nitrate technique have significantly reduced sample preparation time. The use of the automated headspace sampler coupled to the CF-IRMS system has also reduced the amount of nitrate necessary per analysis. Using this method, we are able to analyze samples with nitrate concentrations as low as 0.2 mg/L. The reproducibility of the method is ±0.2‰ for $\delta^{15}N$ and ±0.3‰ for $\delta^{18}O$ (1σ).

The method has been successfully used to identify the sources of vadose zone nitrate at the Hanford site. The relationships between the vadose zone nitrate and nitrate contamination in the groundwater are summarized below.

SIGNIFICANCE OF FINDINGS

The isotopic compositions of nitrate in unsaturated zone (UZ) and groundwater samples from Hanford indicate at least four potential sources of nitrate in the groundwater. Natural sources of nitrate include microbially produced nitrate from the soil column ($\delta^{15}N$ of 4 to 8‰, $\delta^{18}O$ of -9 to 2‰) and nitrate in buried caliche layers ($\delta^{15}N$ of 0 to 8‰, $\delta^{18}O$ of -6 to 42‰). Industrial sources of nitrate include nitric acid in low-level wastewater ($\delta^{15}N$ ~0‰, $\delta^{18}O$ ~23‰) and nitrate in high-level radioactive waste from plutonium processing ($\delta^{15}N$ of 8 to 33‰, $\delta^{18}O$ of -9 to 7‰). The isotopic compositions of nitrate in 97 groundwater wells with nitrate concentrations up to 1,290 mg/L have been analyzed (Figure 1). These data indicate that the primary sources of nitrate in groundwater are nitric acid and natural nitrate flushed out of the UZ during disposal of low-level wastewater. Nitrate associated with high-level radioactive UZ contamination does not appear to be a major source of groundwater nitrate.

RELATED PUBLICATION

Singleton, M.J., K.N. Woods, M.E. Conrad, D.J. DePaolo, and P.E. Dresel, Tracking sources of unsaturated zone and groundwater nitrate contamination using nitrogen and oxygen stable isotopes at the Hanford Site, Washington. Environ. Sci. Technol., 39, 3563–3570, 2005. Berkeley Lab Report LBNL-57044.

ACKNOWLEDGMENTS

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